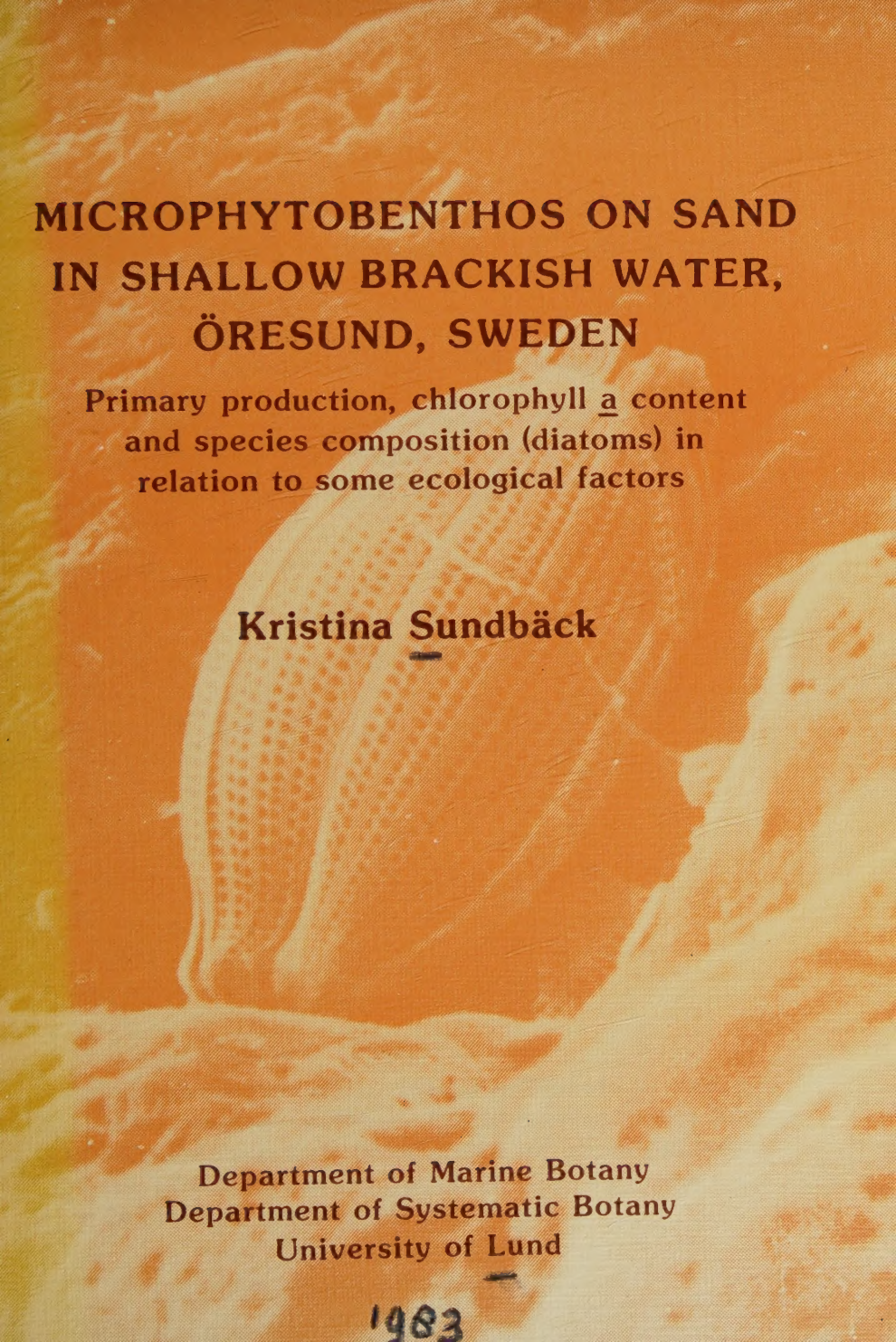


The background of the cover is a high-magnification, orange-toned micrograph of a diatom frustule. The frustule is oval-shaped and shows a complex, symmetrical pattern of pores (pore fields) arranged in a grid-like fashion. The overall color is a warm, monochromatic orange.

MICROPHYTOBENTHOS ON SAND IN SHALLOW BRACKISH WATER, ÖRESUND, SWEDEN

**Primary production, chlorophyll a content
and species composition (diatoms) in
relation to some ecological factors**

Kristina Sundbäck

**Department of Marine Botany
Department of Systematic Botany
University of Lund**

1983

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Kristina Sundbäck
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CORRECTIONS *

Page	Line	
26	4	for 'correction' read 'correlation'
26	10	read 'and the values from Sturup were compared by eye and if agreement was good the tables were used'
28	Table 3	missing first line should read 'Station 1' and 'Station 2'
35	37	for '0.11 $\mu\text{g g}^{-1}$ ' read '0.11 $\mu\text{g g}^{-1}$ '
37	14	omit 'year ⁻¹ '
59	Appendix	missing first line in the table should read 'Station 1' and 'Station 2'
64	28	for 'on tidal' read 'on a tidal'
64	30	for 'important' read 'import'
64	32	for 'Carlberg, R.' read 'Carlberg, S.R.'
65	31	for 'Havsundersö' read 'Havsunders.'
65	38	for '259-256' read '249-256'
66	8	for 'methology' read 'methodology'
66	30	for '192' read '1952'
66	37	for 'at diatom' read 'of diatom'
68	27	for '105' read '106'
85	Table 5	First vertical line from left should be between numbers 2 and 3 on top line
86	11	read ' such as <i>Achnanthes hauckiana</i> , <i>Navicula cryptolyra</i> , <i>Navicula</i> cf.'
95	1	for 'Life forms' read 'Life form'
109	29	for 'on estuary' read 'in an estuary'
116	6-7	read 'Photos a,b and d by H. Håkansson and K. Sundbäck and c by P. Sundström.'
122	48	for 'microscop' read 'microscopic'
126		missing references should read ' Wolf, de H. 1982: Method of coding of ecological data from diatoms for computer utilization.- Med. Rijks Geol. Dients 36:95-98. Öresundskommissionen 1980: Öresund. Tillstånd- -effekter av närsalter.- 252 pp. Stockholm.'
134	Fig. 2.	for ' II' read ' ≡ II'
139	12	for 'on type III' read 'on type IV'
143	6	for 'mixed sand' read 'type II'
145	20	for '(e) epipsammic' read '(e) large attached'
146	Table 5	for missing value, right column read '8.2'

* Obvious misspellings are not included

148 Fig. 7. see correction for p. 134
 159 19 for 'Höisater' read 'Höisaeter'
 162 19 for 'in many' read 'in my'
 163 13 for '1982' read '1979'
 164 14 read 'A. sp. 1 (see Sundbäck, 1983b)
 164 23 read 'C. sp. 1 (see Sundbäck, 1983b)
 165 23 read 'N. sp. 1 (see Sundbäck, 1983b)'
 165 25 read 'N. sp. 5 (see Sundbäck, 1983b)'
 168 24 for 'and environment' read 'and their environment'
 171 32 for 'discharge?' read 'discharge of nutrients?'
 192 7-8 for ':spectrophotometric' read '.Spectrophoto-
 metric'
 197 10 omit lines 10 to 19
 208 10 read 'A. sp. 1 (see Sundbäck, 1983b)
 209 6 for '(Ehrenberg)' read 'Ehrenberg'
 209 16 read 'N. sp. 1 (Sundbäck, 1983b)

MICROPHYTOBENTHOS ON SAND IN SHALLOW BRACKISH WATER, ÖRESUND, SWEDEN

Primary production, chlorophyll a content
and species composition (diatoms) in
relation to some ecological factors

Kristina Sundbäck



LUND 1983

MICROPHYTOBENTHOS ON SAND
IN SHALLOW BRACKISH WATER,
ÖRESUND, SWEDEN

Primary production, chlorophyll *a* content
and species composition (diatoms) in
relation to some ecological factors

Kristina Sundbäck



Cover photo: *Amphora* specimen on a sand grain
(H. Håkansson and K. Sundbäck)

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Abstract Seasonal and spatial variations in the microphytobenthos at two sandy inshore sites were studied during c. 3.5 years. The following environmental variables were measured: temperature, insolation, salinity, pH, grain size distribution, loss on ignition and inorganic P and N of the interstitial water. Simple and multiple correlation analyses were used to extract the most important controlling factors. Numerical analyses (classification and ordination) were applied to extract the main trends of variation in the species composition of benthic diatom assemblages. Experiments were set up to investigate the role of nutrient availability and grazing as controlling factors.			
Conclusions: The microphytobenthos of sandy bottoms can significantly contribute to primary production in shallow coastal waters of SW Sweden. Both production and chlorophyll <u>a</u> content showed clear seasonal variation. Temperature was found to be more limiting than light. The microbenthic flora consists mainly of small (cell length 5--10 μ m) diatoms attached to sand grains (epipsammic) and is characterized by only slight structural changes during the year. Diversity and evenness are high and stable. Spatial variation is related to degree of exposure to water movement; motile species and those that are not firmly attached are fewer in the more exposed habitats.			
As with phytoplankton the microphytobenthos can be nitrogen limited and then assimilates N from the overlying water. In eutrophicated areas the benthic microflora prevents the release of P from the sediment to the water by keeping the sediment surface oxygenated.			
The amphipod <u>Bathyporeia pilosa</u> may occasionally limit the microphytobenthos by grazing.			
Key words microphytobenthos, diatom, primary production, chlorophyll <u>a</u> , epipsammic, numerical analysis, grazing, nutrient, brackish, sediment			
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MICROPHYTOBENTHOS ON SAND IN SHALLOW BRACKISH WATER,
ÖRESUND, SWEDEN

Primary production, chlorophyll a content and species
composition (diatoms) in relation to some ecological
factors

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The dissertation is a summary of the following papers:

- I Sundbäck, K. 1983a: Seasonal variation in microphyto- 15
benthic primary production and chlorophyll a content
on a sandy coast with shallow brackish water, S Öre-
sund, Sweden.- Manuscript.
- II Sundbäck, K. 1983b: The species composition of benthic 67
diatom assemblages on a sandy coast with shallow
brackish water, S Öresund, Sweden.- Manuscript.
- III Sundbäck, K. 1983b: Spatial variation in chlorophyll a 127
content and species composition of diatom assemblages
in relation to sediment characteristics in a non-tidal
shallow bay, S Öresund, Sweden.- Manuscript.
- IV Granéli, E. and Sundbäck, K. 1983: The response of plank-193
tonic and microbenthic algal assemblages to nutrient
enrichment in shallow coastal waters, SW Sweden.
- Manuscript.
- V Sundbäck, K. and Persson, L.-E. 1983: The effect of graz-169
ing by an amphipod, *Bathyporeia pilosa* Lindström on
microphytobenthic biomass and primary production.
- Manuscript.

The different papers will be referred to in the summary by
their Roman numerals.

INTRODUCTION

The south-west coast of Sweden is characterized by shallow virtually non-tidal waters and sandy bottoms. Investigations on the importance of microbenthic primary production from other shallow areas such as tidal flats (e.g. Gröntved, 1962; Pamatmat, 1968; Cadée and Hegeman, 1974) and the Danish coast of Öresund (Gargas, 1970) suggested the subject for my research. I began at a time (1975) when virtually nothing was known about the primary producers of sediments in shallow coastal waters in Sweden. The primary objectives were therefore to estimate the level of primary production, to identify the dominating algal species of the microphytobenthos and to find the main factors controlling seasonal variation (I and II). Öresund (the Sound), between Denmark and Sweden, was considered a suitable study area since measurements of phytoplankton primary production (Edler, 1977) and of the load of nutrients were already being carried out in Öresund by Denmark and Sweden in cooperation (Öresundskommissionen, 1980). The macroalgae of Öresund had been previously investigated by von Wachenfeldt (1975).

Since I began my investigation many papers on microphytobenthos in other shallow water areas, mainly tidal, have appeared. However, since conclusions reported for tidal areas may not be wholly applicable to Swedish coasts, there being virtually no regular tidal exposure with subsequent mixing of the substrate and the overlying water, I therefore continued to collect local background information, for instance on factors controlling spatial distribution of the microphytobenthos (III). Experiments were set up to investigate the role of other possible controlling factors such as nutrient availability (IV) and grazing (V).

STUDY AREA

Öresund is one of the three straits joining the brackish waters of the Baltic with the more saline waters of the Kattegat and is thus characterized by horizontal and vertical salinity gradients. Tidal amplitude is < 10 cm. For further details of hydrography see Öresundskommissionen (1980) and von Wachenfeldt (1975).

Since it was necessary to have access to a laboratory in the vicinity of the sampling sites the field investigations (I, II and III) were concentrated to the Falsterbo peninsula at the southern entrance to Öresund where the Kämpinge Research Station (National Swedish Environment Protection Board) on the Falsterbo canal was placed at my disposal. For details of the hydrography of this area see Jansson et al. (1982). The nutrient-enrichment experiments (IV) were carried out on material from the Falsterbo canal, which is not directly influenced by discharge, and from Lomma Bay, which receives effluents from a tertiary treatment plant for municipal sewage. Sediment and amphipods for the grazing experiments (V) were collected from a shallow sandy bay in the middle part of Öresund.

MATERIAL AND METHODS

Seasonal variation in the microphytobenthos (I and II) was studied during two separate periods: October 1975 to June 1978 (Period I) and April to November 1980 (Period II). Samples were taken once or twice a month at two sandy sites in shallow water (< 1 m): station 1, situated in the basin at the southern entrance to the Falsterbo canal and exposed to swell from canal traffic; station 2, situated immediately west of the northern basin in a more sheltered position. Samples were taken from the top 5 mm of the sediment with a piston corer. Biomass was measured as chlorophyll *a* content, and primary production as assimilation of $H^{14}CO_3$ in sediment subsamples placed in glass bottles incubated in situ for 4 hours. Experiments were set up in the laboratory in which different incubation techniques were compared so that the amount of overestimation induced by the method could be calculated. The following environmental variables were measured during the investigation: temperature, insolation, salinity,

and pH, and during Period II grain size distribution, loss on ignition and concentrations of inorganic nitrogen and phosphorus in the interstitial water were also measured. The density of the macrofauna was also estimated.

Spatial distribution of chlorophyll a content and species composition of diatom assemblages in relation to sediment characteristics were studied by sampling at 50 points within an area of 200 x 200 m (III).

Simple and multiple correlation analyses were used to extract the most important controlling environmental factors for both seasonal and spatial variation.

Since diatoms accounted for the main part of the benthic microflora the quantitative identification of species was restricted to diatoms (II). Relative abundance was calculated from 350--500 valves per slide along central linear transects on permanent slides prepared from oxidized diatoms. Numerical computer-assisted methods (classification and ordination) were applied to extract the main trends of variation.

The usefulness of glass as an artificial substrate for studying the microflora of sandy sediment was tested in a colonization experiment during Period II: Microscope slides attached to a PVC frame were pressed into the sediment and immersed for 4 and 8 weeks (II).

Enrichment experiments (nitrogen and phosphorus) were performed both in the field and in the laboratory using containers with sediment and the natural algal flora (IV). Chlorophyll a content and ^{14}C assimilation were used as a measure of the response of the algae to additions of different concentrations of N and P. All experiments included containers with phytoplankton to compare the response to and uptake rates of nutrients by planktonic and benthic microalgae.

Grazing effect was studied by introducing different densities of the amphipod *Bathyporeia pilosa* into aquaria containing sandy sediment with its indigenous epipsammic microflora (V). Chlorophyll a content and primary production (^{14}C uptake) was measured weekly. Selectivity was studied by comparing the diatom composition in the sediment, stomach contents and faecal pellets.

RESULTS

Seasonal variation in primary production and chlorophyll *a* content (I)

In general chlorophyll *a* values were high in summer and autumn (June-- November) and low during winter, ranging from 1 to 150 mg m⁻². No clear variation of chlorophyll *a* content was observed from year to year. Primary production varied between 4 and 1240 mg C m⁻² day⁻¹ with the highest values being found during the period June to August. The photosynthetic rate was 0.11--1.95 mg C mg chl *a*⁻¹ h⁻¹. The annual production values (corrected for overestimation by incubation method used) ranged between 19 and 67 g C m⁻² year⁻¹ according to station and year.

Both primary production and chlorophyll *a* content were twice as high at station 2 than at station 1 as a result of difference in degree of exposure to water movement.

All correlation analyses point to temperature as the main controlling factor; it accounts for c. 60--75% of the variation in primary production. An increase of 1°C corresponded to an increase of 15-17% in primary production (simple regression).

Species composition (II)

The microbenthic flora was composed mainly of pennate diatoms; cyanophytes and green monads were observed periodically, especially at the more sheltered site (station 2). In all, 145 diatom taxa were identified. Those classified as epipsammic made up 70--80% of the total cell volume of diatoms; they were small (cell length 5--10 μm). Identification was often problematic.

In contrast to seasonal variation in primary production and chlorophyll *a* content only minor variations were observed in the species composition of the diatoms, indicating that the bulk of the diatom flora was made up of the same attached diatoms all year round. The stableness of the diatom flora was combined with stable and high diversity ($H' = 3.28--4.55$) during the whole investigation.

Classification separated out the species composition of the two stations as two clusters: an *Achnanthes hauckiana*-association (station 1) and a *Fragilaria pinnata*-*Opehora martyi*-association (station 2). The difference between these associations

was quantitative rather than qualitative. In an attempt to further analyse the distinction between the associations the diatom taxa were divided into 5 classes according to life form based on mode of attachment, motility and size. Station 1 was dominated wholly by flat life forms attached to cavities in the sand grains, whereas protruding and colony-forming life forms were co-dominants at station 2. This difference is interpreted as a result of degree of exposure to water movement, a higher degree of exposure giving rise to a flora consisting almost entirely of diatoms that are firmly attached to the sand grains or that are protected within the cavities. This also explains the lower chlorophyll a values at station 1 which is exposed to swell from canal traffic.

The colonization experiment showed that glass slides, although they were submerged in the sediment proved to be a more favourable substrate for epiphytic microalgae than for epipsammic microalgae. In contrast to the diatom flora of the sediment diversity in the diatom flora of the glass slides was characterized by lower and more varying values ($H' = 0.40-4.40$), and there was a clear successional trend owing to an invasion of *Cocconeis placentula*. Although glass is often used for periphyton studies it does not seem to be a suitable substrate for studying the microflora of sandy sediments. Obviously it is a poor imitation of the natural habitat with its abundance of microniches.

Spatial distribution of microphytobenthos in relation to sediment characteristics (III)

Both chlorophyll a content and species composition were correlated with sediment type. Exposure to wave action controlled the amount and type of benthic microflora. Ordination diagrams based on relative abundance showed that variation in species composition was continuous rather than patchy and quantitative rather than qualitative. The proportions of the different life forms changed gradually along the exposure gradient. The continuity was, however, broken in one place where the accumulation of decomposing macroalgae changed chemical conditions in the habitat.

Response of microphytobenthos to nutrient enrichment (IV)

Since the surface of sediments is rich in nutrients they are not generally considered limiting for sediment-associated microflora. Experiments using natural microalgal assemblages from the Falsterbo area, however, showed that microphytobenthic growth was stimulated by nitrogen additions, as was the growth of phytoplankton in the same area (Granéli, 1981). However, in the nutrient-loaded Lomma Bay with its high concentrations of inorganic N, phytoplankton was phosphorus-limited whereas the benthic microflora did not respond to additions of either N or P. On the other hand the benthic microflora controlled the flux of P from the sediment to the water by keeping the sediment surface oxygenated. Added nutrients disappeared from the water faster in the sediment containers than in the phytoplankton containers. Uptake by microphytobenthos explained c. 20% of this loss, the rest probably being accounted for by other processes in the sediment, e.g. denitrification. Using C:N:P ratios from the literature and the amount of carbon fixed during a 23-day experiment, it was calculated that the microphytobenthos assimilated c. 40% of the initial pool of inorganic N in the sediment containers (overlying water + interstitial water); phytoplankton answered for c. 90%.

The effect of grazing on microphytobenthos (V)

Laboratory experiments showed that natural densities of the amphipod *Bathyporeia pilosa* limited biomass (chlorophyll *a* content) and primary production in the natural epipsammic flora. Selectivity for the diatom genus *Cocconeis* was observed in one of the experiments. The results suggest that grazing is a limiting factor and further that migration in *B. pilosa* is partly induced by food limitation (Persson, 1982).

CONCLUSIONS

- (1) The microphytobenthos of sandy bottoms can significantly contribute to primary production in shallow coastal waters of SW Sweden.
- (2) Microbenthic primary production and chlorophyll a content show clear seasonal variation. Temperature was found to be more limiting than light.
- (3) The microbenthic flora consists mainly of small (cell length 5--10 μ m) diatoms attached to sand grains (epipsammic) and is characterized by only slight structural changes during the year. Diversity and evenness are high and stable.
- (4) Spatial variation is related to degree of exposure to water movement; motile species and those that are not firmly attached are fewer in the more exposed habitats.
- (5) As with phytoplankton the microphytobenthos can be nitrogen limited and then assimilates N from the overlying water. In eutrophicated areas the microflora prevents the release of phosphorus from the sediment to the overlying water by keeping the sediment surface oxygenated.
- (6) The amphipod *Bathyporeia pilosa* may seasonally limit the microphytobenthos by grazing.

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REFERENCES

- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea.- *Neth. J. Sea Res.* 8: 260-291.
- Edler, L. 1977: Phytoplankton and primary production in the Sound.- *Doct. thesis, Dept of Marine Botany, University of Gothenburg, Sweden.*
- Gargas, E. 1970: Measurements of primary production, dark fixation and vertical distribution of microbenthic algae in the Öresund.- *Ophelia* 8: 231-253.
- Granéli, E. 1981: Experimental investigations of limiting nutrients for phytoplankton production in the brackish-water Öresund, SW Sweden.- *Doct. thesis, Dept of Marine Botany, University of Lund, Sweden.*
- Grøntved, J. 1962: Preliminary report on the productivity of microbenthos and phytoplankton in the Danish Wadden Sea.- *Medd. Danm. Fiskeri- og Havsunders.* 3: 347-372.
- Jansson, A.-M., Kautsky, N., von Oertzen, J.-A., Schramm, W., Sjöstedt, B., von Wachenfeldt, T. and Wallentinus, I. 1982: Structural and functional relationships in a southern Baltic Fucus ecosystem.- *Contr. Askö. Lab.* 28, 95 pp.
- Pamatmat, M.M. 1968: Ecology and metabolism of a benthic community on an intertidal sandflat.- *Int. Rev. ges. Hydrobiol.* 53: 211-298.
- Persson, L.-E. 1982: Seasonal migration of Bathyporeia pilosa Lindström in the southern Baltic.- *Ophelia* 21: 205-213.
- Wachenfeldt, von T. 1975: Marine benthic algae and their environment in the Öresund.- *Doct. thesis, Dept of Marine Botany, University of Lund, Sweden.*
- Öresundskommissionen 1980: Öresund. Tillstånd - effekter av närsalter. - 252 pp. Stockholm.

SEASONAL VARIATION IN MICROPHYTOBENTHIC PRIMARY
PRODUCTION AND CHLOROPHYLL A CONTENT ON A SANDY
COAST WITH SHALLOW BRACKISH WATER, S ÖRESUND,
SWEDEN

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ABSTRACT

Seasonal variation in microphytobenthic primary production (^{14}C fixation) and chlorophyll a content in the top 5 mm of sediment was studied at two sandy sites in shallow water. Samples were taken once or twice a month during two periods: October 1975 to June 1978, and April to November 1980. The environmental variables considered were: water temperature, solar radiation, pH and salinity. During the second period, in addition, some physico-chemical characteristics of the sediment were measured, e.g. grain size distribution, loss on ignition and inorganic nutrients of the interstitial water. Primary production varied between 4 and 1240 $\text{mg C m}^{-2} \text{ day}^{-1}$, the highest values being found during the period June to August (summer) and low values during winter. Annual values for primary production varied between 19 and 67 $\text{g C m}^{-2} \text{ year}^{-1}$ and the photosynthetic rate between 0.11 and 1.95 $\text{mg C mg Chl a}^{-1}$. Chlorophyll a content varied between 1 and 154 mg m^{-2} with the highest values during the period June to November. There was no clear year-to-year variation in chlorophyll a content. Both chlorophyll a and primary production values were twice as high at the one site than at the other. This is believed to be a result of difference in degree of exposure to water movements. In all 23 biotic and abiotic variables were used in single and multiple correlation and regression analyses to describe the relationship between microphytobenthos and environmental variables. All analyses extracted temperature of water as the main controlling factor explaining 60-75% of the variation in daily primary production. The correlation between chlorophyll a content and temperature was weaker.

1. INTRODUCTION

Öresund (the Sound) situated between SW Sweden and Zealand, Denmark, is a brackish strait with extensive areas of shallow water with sandy bottoms. Aerial photos show that about 70% of the sea floor between 0 to 5 m consists of sandy sediments with no macroflora (von Wachenfeldt, 1978). This fact and reports on the importance of microbenthic primary production from other shallow areas such as tidal flats (Grøntved, 1960, 1962; Pamatmat, 1968; Cadée and Hegeman, 1974), prompted this investigation of the microbenthic flora in Öresund.

The aim was to get a basic estimate of the level of primary production and the trend of seasonal variation. Gargas (1970) measured the microphytobenthic production in the shallow Nivå Bay on the Danish coast of the Sound, but his measurements were made during only one season.

My investigation was started at a time when few results that took into account both production and the structure of the microphytobenthic community had been published. Therefore it was felt that there was a need to combine both aspects in a single investigation, so that both spatial and temporal variations and the major controlling factors could be better understood. The greater interest displayed in the microphytobenthos during the mid-seventies resulted in many integrated investigations. Of these the extensive investigations from the Dutch Wadden Sea, and in particular from the Ems-Dollart estuary, have given much information (e.g. Colijn and Koeman, 1975; Colijn and Dijkema, 1981, Admiraal, 1980; review by van den Hoek et al., 1979).

Since a laboratory in the vicinity of the sampling stations was an absolute necessity, the investigation was concentrated to the Falsterbo peninsula at the southern entrance of Öresund, where the Kämpinge Research Station (National Swedish Environment Protection Board) was placed at my disposal.

The aims were:

- (a) to estimate the primary production of sandy inshore bottoms in southern Öresund;
- (b) to study the major controlling factors of seasonal variation by correlating environmental variables and

(c) to describe the structure of the microphytobenthic community (species composition).

The investigation was to serve as a starting point for a further study of controlling factors such as salinity, nutrients and biological interactions and to provide reference material for assessing the effect of artificial eutrophication and other results of human activity in Öresund. For convenience production measurements and species composition will be presented separately (Sundbäck, 1983a).

2. MATERIAL AND METHODS

2.1. The study area

The investigation was carried out at two localities on the Falsterbo peninsula (Fig. 1). Öresund, which joins the Baltic Sea with the Kattegat, is characterized by a horizontal salinity gradient. The tides in the area are negligible. Fluctuations in water level are mainly due to local differences in air pressure and currents. The hydrography of Öresund is described in Öresundskommissionen (1980).

Both sampling stations are on sandy sediment in shallow water (<1m) but differ in degree of exposure to water movement (see 3.2.). Station 1 is situated at the southern entrance to the Falsterbo canal (Fig. 1) in an artificial basin formed by stone breakwaters, but is influenced by canal traffic. Station 2 is situated immediately to the west of the northern basin at the entrance to the Fälsterbo canal, c. 2 km from station 1 (Fig. 1). The physico-chemical characteristics of the sampling stations are described in greater detail in 3.2.

2.2. Sampling

The investigation comprised two periods: October 1975 to June 1978 (Period I) and April to November 1980 (Period II). Sampling was done biweekly to monthly, more frequently during Period I than during Period II. During July-August in 1976

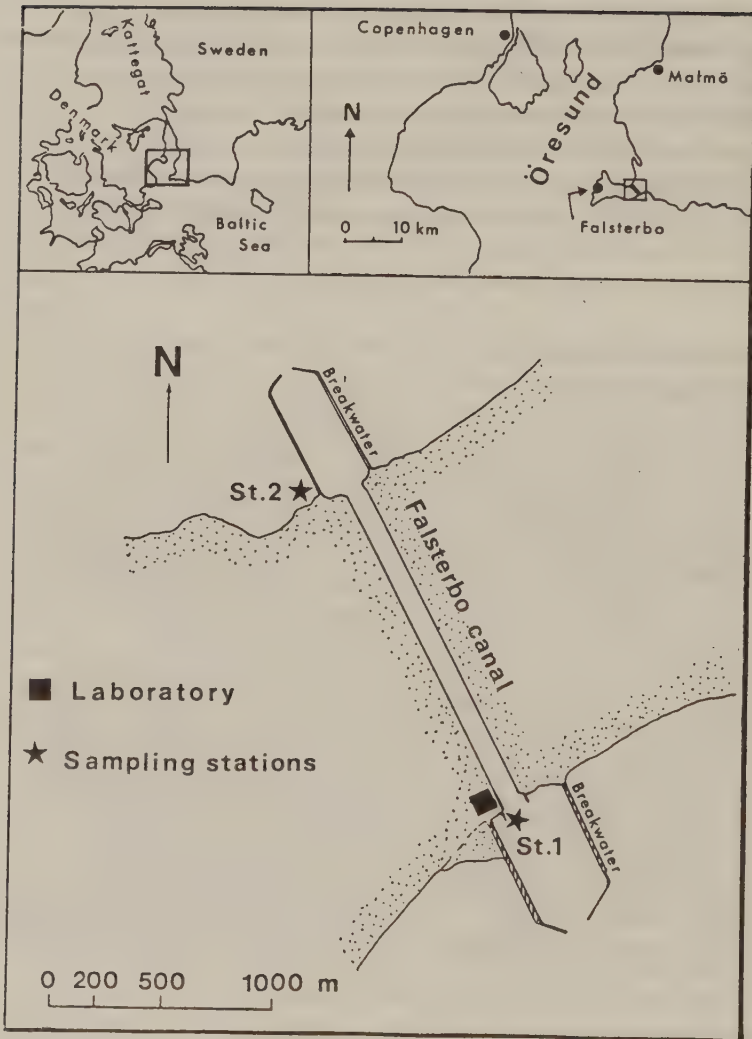


Fig. 1. Location of the two sampling stations on the Falsterbo peninsula, SW Sweden.



Fig. 2. Piston corer used during investigation Period I. Modified after Gargas (1970).

samples were taken at intervals of 2 to 6 days. In all station 1 was sampled 66 times (days) and station 2 49 times. Table 1 indicates variables measured during Periods I and II. The structure of the natural microflora of the sediment and of the microflora colonizing glass slides were also compared during Period II. The species composition will be presented in a separate article (Sundbäck, 1983a).

During Period I sediment samples of 3.14 cm^2 were taken with a plexiglass tube (2 cm ID) according to the method described by Gargas (1970) but with the following modifications: an inner PVC tube with a rubber stopper was used as a piston, to slowly lower the sediment core so that the top 5 mm could be cut off with the knife through the slit (Fig. 2). During Period II the tip of a 2 ml plastic syringe (9 mm ID) was cut off and the syringe was used as a corer. Three samples of 0.64 cm^2 were pooled and analysed as one sample. The advantages of using this sampling technique were: (a) easier and quicker sampling, (b) the effect of patchy distribution of the microflora could be diminished by pooling samples. Four samples were taken for chlorophyll a analyses, 2-4 samples for primary production measurements and 2-4 samples for species composition.

Table 1. Units and abbreviations of variables measured during Period I (October 1975 - June 1978) and Period II (April - November 1980).

Variable	Abbreviation	Unit of measurement	Period I	Period II
Chlorophyll <u>a</u>	Chl	mg m ⁻²	x	x
Pheopigment	Pheo	mg m ⁻²	x	x
Primary production per incubation time	ProInc	mg C m ⁻² 4 h ⁻¹	x	x
Daily primary production	ProDay	mg C m ⁻² day ⁻¹	x	x
Dark fixation	DarkFx	mg C m ⁻² h ⁻¹	x	x
Assimilation number	AssNo	mg C mg chl ⁻¹ h ⁻¹	x	x
Temperature	Temp	°C	x	x
Daily solar radiation	LiDay	E m ⁻² day ⁻¹	x	x
Solar radiation during incubation	LiInc	E m ⁻² 4 h ⁻¹		
Accumulated solar radiation	LiAcc	E m ⁻² 10 days ⁻¹	x	x
pH	pH		x	x
Salinity	Sal	°/oo	x	x
Wind force*	Wind	m s ⁻¹	x	x
Loss on ignition	Ign	% of dry weight		x
NH ₄ -N	NH ₄	10 ⁻⁶ M		x
NO ₃ + NO ₂ -N	NO ₃	10 ⁻⁶ M		x
PO ₄ -P	PO ₄	10 ⁻⁶ M		x
Proportion sediment particles <0.1 mm	GrA	% of dry weight		x
Proportion sediment particles 0.1 - 0.15 mm	GrB	% of dry weight		x
0.15-0.25 mm	GrC	% of dry weight		x
0.25-0.4 mm	GrD	% of dry weight		x
>0.4 mm	GrE	% of dry weight		x
Median grain size	MedGr	mm		
Sorting coefficient	Sort	σ _g		x
Macrofauna*	Fauna	indiv. m ⁻²		x

* Variable not used in statistical calculations.

Overlying water was sampled in 1-liter plastic flasks for pH measurements and salinity and for use in ^{14}C bottles. Samples for sediment analysis and macrofauna were taken with a PVC tube (7 cm ID) down to 4 cm sediment depth: 3 samples for nutrient analyses of the interstitial water, 3 samples for mechanical analyses and loss on ignition and 2-4 samples for macrofauna.

Station 1 was sampled within a sandy patch c. 4 x 5 m between a small stone jetty and a patch of *Fucus vesiculosus*. Thus the deviation of the sampling point from time to time was negligible. At station 2, however, the sampling point varied from time to time with c. 20 m during Period I, because it was not permanently marked. From October 1976 to June 1978 (19 times) two points were sampled at station 2, situated c. 40 m apart. During Period II (1980) the sampling point at station 2 was permanently marked and situated c. halfway between the two previous points.

The samples were placed in a thermally isolated box and transported to the nearby Kämpinge Research Station. Analyses were begun not more than one hour after sampling.

2.3. Treatment of samples

Primary production was measured as H^{14}CO_3 uptake according to the principle of Steemann-Nielsen (1952) and modified for microphytobenthos by Grøntved (1960), Gargas (1970) and Cadée and Hegeman (1974), etc. However, in this investigation no attempt to separate the epipsammic and suspendible fractions was made first.

A subsample of c. 0.1 cm^3 was taken from the homogenized sediment sample with a glass tube (5 mm ID) and put into a glass incubation bottle containing 100 ml or 25 ml glass-fiber-filtered water from the sampling site. $4 \mu\text{Ci NaH}^{14}\text{CO}_3$ (^{14}C -Agency, Hörsholm, Denmark) was added to each bottle. The bottles were incubated in situ between 10 a.m. and 2 p.m., usually 2-4 light bottles and one dark bottle at each station. The bottles were anchored so that they were just

touching the sediment surface but could move as the water moved. During the incubation the bottles were not hanging upright so that the sediment particles were not spread out in a single layer over the whole of the bottom of the bottle.

After incubation the samples were immediately collected (vacuum not exceeding 0.5 atm) on Sartorius membrane filters and were exposed to fumes of formalin and HCl for 10 minutes. The filters were air dried and weighed to correct for inaccurate subsampling. The amount of fixed ^{14}C was measured in a Geiger-Müller counter in two fractions: the sand grains were scraped off the filter and the filter and sand were measured separately. The sand grains were spread out in a single layer on a small aluminium plate and the netto cpm (corrected for dark fixation and background) was multiplied by 2 according to Gröntved (1960), a method which Cadée and Hegeman (1974) found to be correct. The cpm values were converted to $\text{mg C m}^{-2} \text{ h}^{-1}$ using measurements of pH, temperature and salinity (for calculating CO_2 concentration) and the formulae in Gargas (1975). The production per day was calculated according to Schindler and Holmgren (1971) using insolation values from the meteorological station at Sturup (see below).

To estimate the effect of incubation method on the primary production values I twice compared three different techniques in the laboratory: (a) method used here (also by Gröntved, 1960; Cadée and Hegeman, 1974, 1977) (b) a small intact core placed in a cup in an incubation vessel (Marshall et al. 1973; Cadée and Hegeman, 1974; Joint, 1978) and (c) a sediment core sampled with a transparent tube stoppered at the lower end and replaced in the sediment for incubation (Leach, 1970; Colijn, 1978; Rasmussen et al. 1983) (Fig. 3). All methods were tested in triplicate.

Chlorophyll a content was measured according to Lorenzen (1967) after 1 hour extraction with 90% acetone in a refrigerator. The flasks were vigorously shaken by hand every 10 minutes during extraction. Absorption coefficient 11.4 was used for chlorophyll a content (Coveney, pers. comm.).

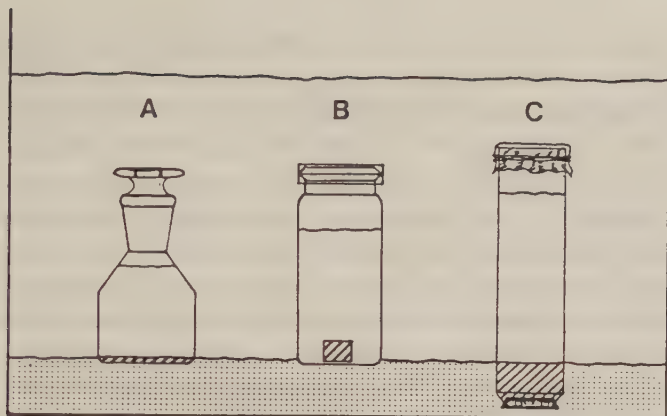


Fig. 3. Experimental design of the incubation method test in laboratory. The inclined flasks used in Experiment B have not been drawn (see 3.5.).

Interstitial water for the analyses of inorganic nutrients ($\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{+NO}_2\text{-N}$) were extracted by centrifuging (3000 rpm, 10 minutes) a subsample from three pooled and mixed samples. Analyses were carried out manually on unfixed samples according to the methods in Carlberg (1972). During autumn 1980 the nutrients in the overlying water were also measured.

Mechanical analyses of the uppermost 4 cm of sediment were made by sieving dry sediment through 0.5, 0.4, 0.25, 0.1 and 0.063 mm screens. Median grain size and sorting coefficient were determined graphically from cumulative curves (e.g. Gray 1981). The organic content of the sediment was measured as loss on ignition at 550°C for 2 hours.

The macrofauna was assessed on seven sampling occasions so that the possible grazing effect on the microflora could be roughly estimated. The macrofauna were counted and identified by Dr L.-E. Persson, Department of Zoology, Animal Ecology, University of Lund.

Temperature was measured in situ at the sediment surface with a mercury thermometer to the nearest 0.5°C . Salinity (conductivity meter) and pH were measured in a water sample

taken close to the sediment surface. When a portable pH-meter was available pH was measured in situ at the sediment surface and a few millimetres below the surface. The pH of the overlying water was used for curves and correction analyses.

Insolation values from two sources were used: a solarimeter on the roof of the laboratory at station 1 and tables from the meteorological station at Sturup (30 km from the investigation area) provided by the Swedish Meteorological and Hydrographical Institute (SMHI). The solarimeter curve and the values from Sturup tables were used. The original unit of the tables (mWh cm^{-2}) was converted to E m^{-2} for wave lengths of 380-700 nm (PAR) using Jerlov's (1974) conversion factor. Force and direction of the wind for Falsterbo were obtained from SMHI.

2.4. Statistical analyses

In all 23 variables (11 of them only for Period II, Table 1) were used in correlation and regression analyses using mean values of each variable on each sampling date. The data set was divided according to station, year, and/or season. The following BMD-programs (Dixon, 1981) were used: 1D (simple data description), 6D (bivariate scatter plots, simple linear regression), 1R (multiple linear regression), 2R (stepwise regression), 3S (nonparametric statistics: Spearman rank correlation). Computations were made at the Lund University Computing Centre. For further details see 3.6.

3. RESULTS

3.1. Hydrography and weather conditions during the investigation

A general description of the climate in the Falsterbo area is given by von Wachenfeldt in Jansson et al. (1982). Figures 4 and 5 (fourth curve from the top) show the photosynthetic active radiation (PAR) on sampling days (LiDay) and the mean value for 10 days preceding the sampling day (LiAcc). The incubation was started at c. 10 a.m. but in 1980 actually one hour earlier, because summertime was introduced in Sweden that year. This deviation was, however, negligible according to the solar radiation tables.

Since photosynthesis is a photochemical process it may be assumed that if sampling during one year often coincides with cloudy weather this would be reflected in the annual production curve. If the solar radiation values for incubation days for the different years are compared (only period April-November considered), the mean value for 1976 is 11% lower than for 1977 and 7% lower than for 1980. The penetration of light to a depth of 2 mm below the sediment surface was measured once at station 1. The photocell of a quantameter, with a 2 mm rim, was covered with 2 mm of sediment. The results (Table 2) agree with the more sophisticated measurements by Fenchel and Straarup (1971) and Haardt and Nielsen (1980).

Table 2. Amount of light penetrating the sediment to a depth of 2 mm at station 1

	% of light	
	at sediment/water interface	at air/water interface
Blue light	0.3	0.2
Green light	0.6	0.4
Red light	2.6	1.5

The temperature at the sediment surface varied between 0.5 and 29°C on the sampling days (Figs 4 and 5). During the 1976-77 winter station 2 was at times covered by ice. Station 1 had no ice-cover on sampling dates, but drifting ice occurred occasionally.

The salinity varied between 7.2 and 15.4 ‰ and was slightly higher at station 2 facing Öresund than at station 1 facing the Baltic (Table 3, Figs 4 and 5). The salinity may fluctuate rapidly due to changes in the speed and direction of the currents (von Wachenfeldt, 1975) and the fluctuation range may be 7-28‰ in surface water c. 10 km north of the study area (Jansson et al., 1982). The general annual trend is towards high salinity during autumn and low salinity in spring with

Table 3. Characteristics of the two sampling stations. Mean value and range (in brackets) shown. For number of observations and coefficient of variation see Appendix 1.

Exposure to wind, direction	SSE - E	W - NW
Exposed to swell from canal traffic	yes	no
Depth of water (m)	0.35 (0-0.6)	0.3 (0-0.6)
Salinity (‰)	8.4 (7.2-11.4)	9.4 (7.2-15.4)
Median grain size (mm)*	0.255 (0.235-0.28)	0.21 (0.2-0.225)
Sorting coeff. σ_g *	0.45 (0.38-0.47)	0.48 (0.44-0.51)
Loss on ign. (% of dry wt)*	0.2 (0.15-0.23)	0.53 (0.32-0.9)
Nutrients in interst. water:*		
PO ₄ -P, $\cdot 10^{-6}$ M	10.5 (4.7-17.5)	7.3 (3.6-12.5)
NH ₄ -N, $\cdot 10^{-6}$ M	26.5 (14.0-44.8)	32.9 (7.0-181.4)
NO ₃ +NO ₂ -N, $\cdot 10^{-6}$ M	4.6 (1.4-6.8)	4.6 (1.4-8.4)
Macrofauna, indiv. m ⁻² *	1,280 (360-3,300)	3,900 (2,000-9,500)
Macroflora	none	<i>Ruppia</i> sp. and floating filamentous algae

* Measured only in 1980 (Period II).

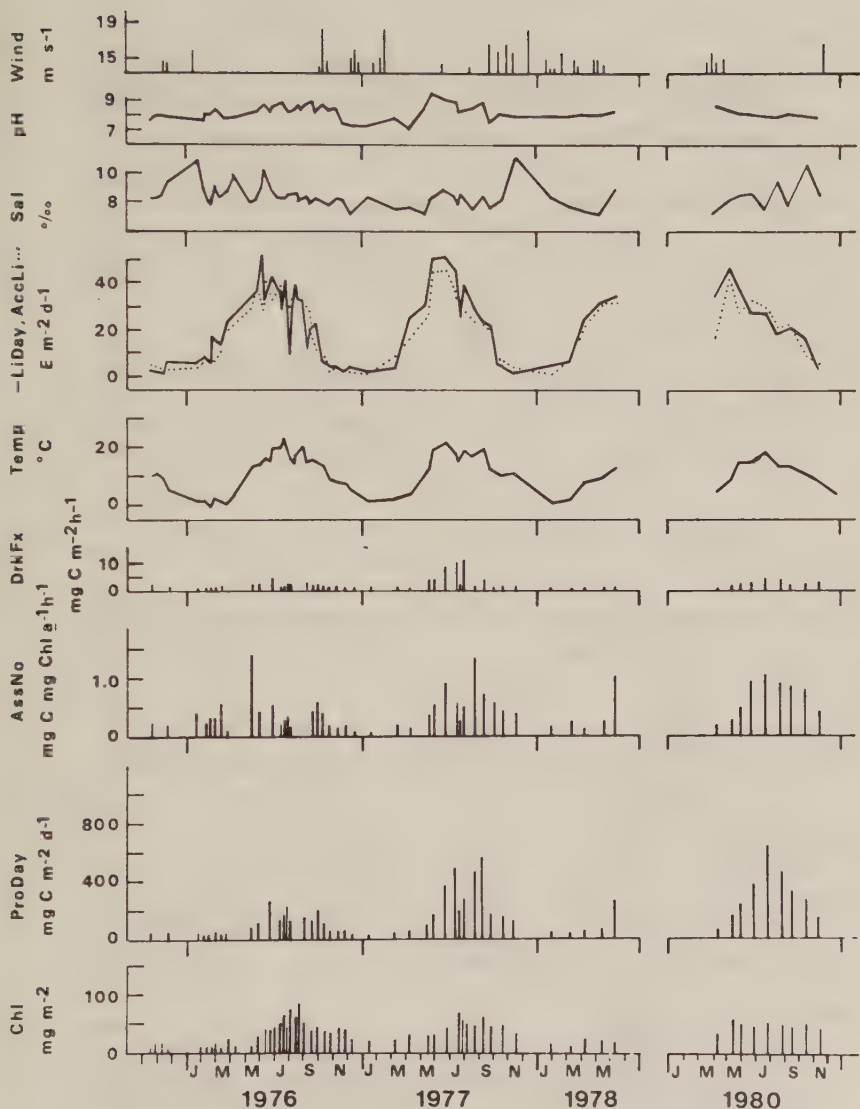


Fig. 4. Variations in production and biomass variables of the microphytobenthos and environmental variables at station 1. For abbreviations see Table 1. Abbreviations of months: J = January, M = March, etc. Note: only alternate months indicated. Only wind forces $>13 \text{ m s}^{-1}$ are shown. AccLi = mean value for 10 successive days.

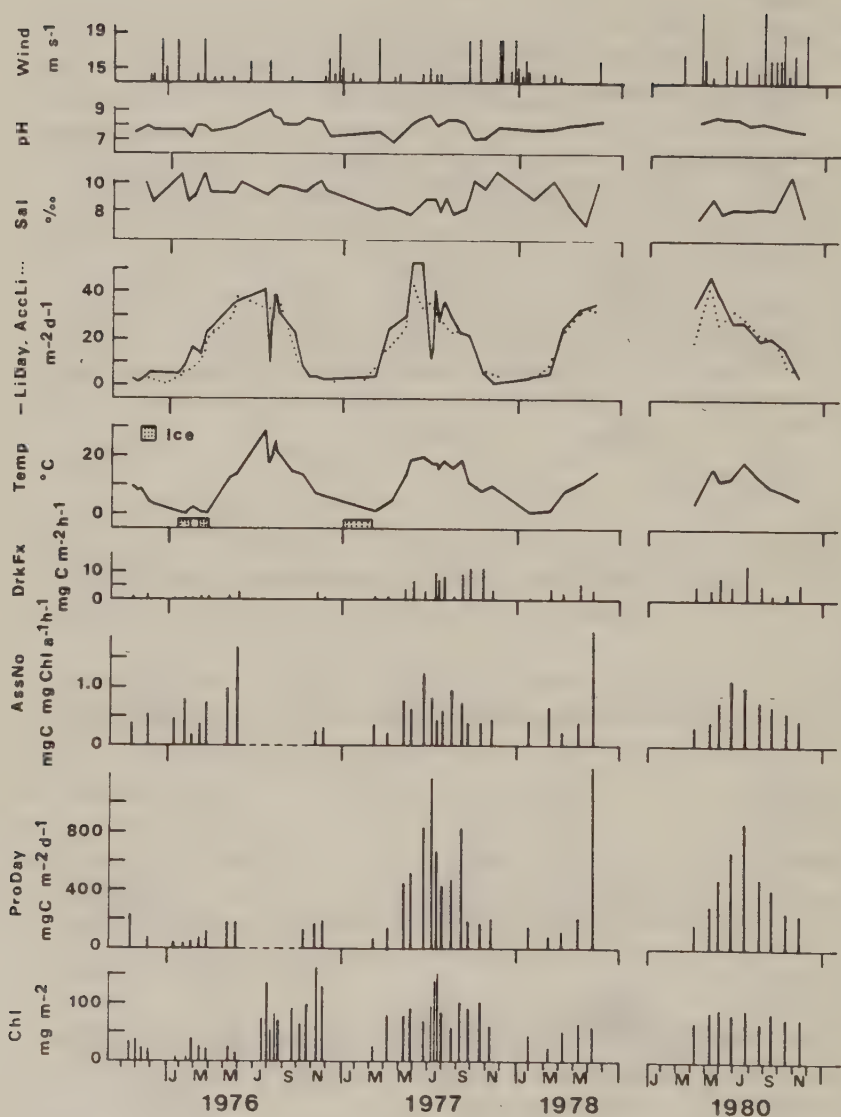


Fig. 5. Variations in production and biomass variables of the microphytobenthos and environmental variables at station 2. For abbreviations see Table 1. Abbreviations of months: J = January, M = March, etc. Note: only alternate months indicated. Only wind forces $>13 \text{ m s}^{-1}$ are shown. AccLi = mean value for 10 successive days.

St.1

St.2

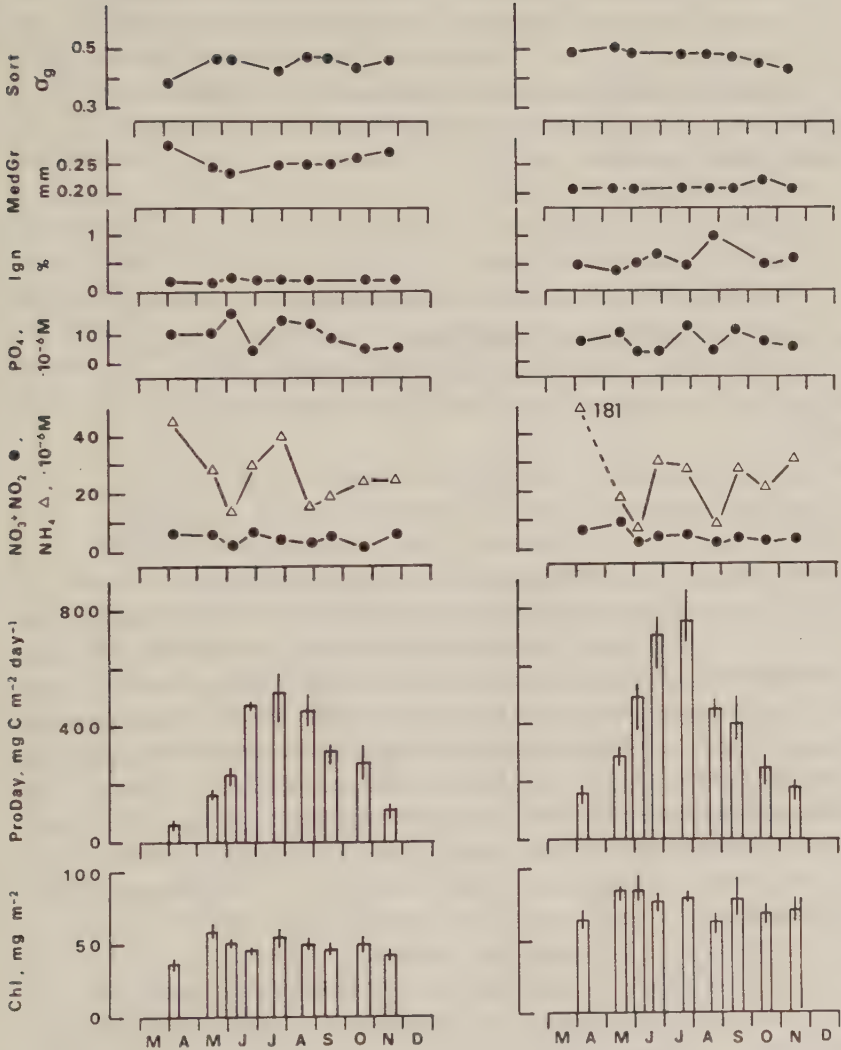


Fig. 6. Variations in microphytobenthic production, chlorophyll *a* content, nutrients and sediment variables at stations 1 and 2 during Period II (1980).

the strongest fluctuations usually occurring in January-February (von Wachenfeldt, 1975) (Figs 4 and 5).

Values for pH at the sediment surface varied between 7.00 (winter) and >9.00 (summer) (Figs 4 and 5, Appendix 1). In situ pH values in the top few millimetres agreed with the values for the water immediately above the sediment. Periods with strong winds occurred mainly during autumn and winter (Figs 4 and 5).

3.2. Sediment characteristics and exposure to water movement.

Some physico-chemical characteristics of the two sampling stations are summarized in Table 3 (see also Fig. 6). Note that most variables were measured only during Period II (1980).

Station 1 is exposed to winds from the southeast (Fig. 1). The samples were taken east of a small stone jetty which affords protection from southerly and southwesterly winds but the site is exposed to heavy swell from the traffic in the canal (boats up to 15,000 tons). The sediment at station 1 consists of pale sand mixed with fragments of shells and can be characterized as well-sorted fine to medium sand (Table 3). Grain-size distribution changes slightly during the year (Fig. 6), the calmer weather conditions prevailing during the summer resulting in a finer sediment. The lower sorting coefficient (=better sorted) found in spring is a result of winter storms (Fig. 6). Loss on ignition was low ($\bar{x}$ 0.20%) and varied little (Table 3, Fig. 6). The density of macrofauna was low (Table 3); *Hydrobia* spp. and *Nereis diversicolor* were the commonest species.

Station 2 is exposed to westerly to northwesterly winds but is more sheltered than station 1 because the waves break a considerable way out and because it is not influenced by swell from the canal traffic. Since the whole area is very shallow, stretches of sand up to 200 m from shore become exposed during periods of extreme low water. The sediment is well-sorted fine greyish-yellow sand (Table 3). Sediment structure does not

change markedly during the year and the median grain size (0.21 mm, CV=2%) was almost constant (Fig. 6). This and the slightly higher sorting coefficient reflect the more sheltered position of station 2. Loss on ignition was c. 0.5% and varied more than at station 1 (Table 3, Fig. 6). Patches of *Ruppia* sp. occurred at station 2. During summer the whole area was occasionally covered by floating filamentous algae. In late summer and autumn decaying macroalgae accumulated near the shore. The presence of purple bacteria and a smell of H_2S indicated anoxic conditions. The sampling points were located outside this area, but purple bacteria were also found on sandbanks c. 200 m from the shore. The density of macrofauna was normal for this shore type (Table 3) (Persson, pers. comm.). *Hydrobia* spp. and *Nereis diversicolor* were the commonest members of the macrofauna.

3.3. Inorganic nutrients

The concentrations of inorganic nutrients in the interstitial water (measured only in 1980) are to be regarded as rough mean values for the top 4 cm of sediment.

The concentrations of phosphorus and nitrogen did not differ between the stations (Tables 3 and 4, Fig. 6). The higher mean value for NH_4 -N at station 2 (see Appendix 1) is due to one extreme value in April (Table 4).

The concentration of PO_4 -P was up to 30 times that of the overlying water, NH_4 -N up to 40 times and NO_3+NO_2 -N up to 8 times (Table 4).

3.4. Chlorophyll a concentration of the sediment

The chlorophyll a concentration was generally low in winter and high in summer and autumn (Figs 4 and 5), varying between 2.4 and 84 $mg\ m^{-2}$ (c. 0.3-11.2 $\mu g\ g^{-1}$ dry sand) at station 1 and between 1 and 154 $mg\ m^{-2}$ (c. 0.1-21 $\mu g\ g^{-1}$ dry sand) at station 2 (Appendix 1). There was no significant difference

in chlorophyll a content between the two sampling points at station 2 during Period I (see Material and Methods). Throughout the investigation the chlorophyll a values for station 2 ($\bar{x}$ 62.4 mg m⁻²) were about twice as high as those at station 1 ($\bar{x}$ 35 mg m⁻²), with one exception, i.e. in winter 1976, when station 2 was covered by ice.

The highest chlorophyll a values at station 1 were found from June to August. At station 2 there were two slight peaks in 1976 and 1977, one in July and one during late autumn. The highest chlorophyll a concentration was found in November 1976 (154 mg m⁻²). During 1980 the chlorophyll a curve was even because of a shorter sampling period and fewer sampling occasions.

There was no clear year-to-year variation in chlorophyll a content. At station 1 the mean values for the period April to November for the years 1976, 1977 and 1980 were 44, 42 and 48 mg m⁻² respectively. Corresponding values for station 2 were 77, 73 and 73 mg m⁻².

In 1976 chlorophyll a samples were taken at intervals of 2 to 6 days from July to August (5 weeks, 10 times) at station 1 and from late July to mid-August (2.5 weeks, 5 times) at station 2. The coefficient of variation (CV, %) for these periods was 19% for station 1 and 38% for station 2. The higher value for variation at station 2 is caused by a drop from 130 mg to 46 mg m⁻² within three days. Since the patchy distribution of benthic microflora is well known (e.g. van den Hoek, 1979) this variation may partly be due to uneven small-scale distribution: the variation between replicate samples ranged between 5 and 30% ($\bar{x}$ 19%, CV). The dramatic drop at station 2 can, however, hardly be the result of a sampling error but may be related to a period with extremely high temperature in the shallow water (up to 29°C) in late July. The spatial distribution of microphytobenthos at station 2 will be further discussed in Sundbäck (1983b).

The concentration of pheopigments varied between 0 and 53 mg m⁻² (c. 0-7 μ g g⁻¹ dry sand) at station 1 and between 0 and 83 mg m⁻² (c. 0 - 11 μ g g⁻¹ dry sand) at station 2 (Appendix 1). The seasonal variations followed the same trend as for chlorophyll a.

3.5. Primary production

The daily primary production varied between 4 and 1240 mg C m⁻² day⁻¹ and followed the same general trend as for chlorophyll a (Figs 4, 5 and 6). The production values varied more than the chlorophyll a values (CV >90% as against 55%, Appendix 1), which is expected since photosynthesis is more likely to be directly influenced by the conditions during the incubation. Primary production was about twice as high at station 2 than at station 1 (cf. chlorophyll a values). Maximum values for the whole investigation (>1000 mg C m⁻² day⁻¹) were measured at station 2 during the period June-July.

The assimilation of ¹⁴C in light was where possible calculated from at least three incubation bottles - the three values varied considerably. For Period I the mean variation was by a factor of 1.4 and for Period II, 1.3.

The separation of the sand from the filter for GM-counting made it possible to estimate the proportions assimilated by attached and unattached organisms. The sand fraction accounted for 85% (CV = 22%) of the total assimilated ¹⁴C at station 1 and 80% (CV = 14%) at station 2. The highest values for the filter fraction (suspensible fraction) were found in early spring, late summer and autumn. The dominance of the epipsammic microflora was confirmed in the species composition analyses (Sundbäck, 1983a).

The first experiment (A) to test the three incubation methods suggested that the method used here gave an over-estimation of the production by a factor of 3 to 5 (Table 5). The second test (B), where inclined bottles more accurately simulated conditions in the in situ experiment, lowered the error to 1.8-3 times. However, ¹⁴C assimilation in the intact cores is probably underestimated, since it was not possible to control that the labelled water actually replaced the initial interstitial water (cf. Colijn and van Buurt, 1975; Revsbech et al., 1981. Note, however, the in situ-method used by Rasmussen et al., 1983, where interstitial water is replaced by natural drainage during low water). This problem applies to method c in particular (Table 5), where water has to be changed carefully so as not to disturb the surface of the

Table 5. Microbenthic primary production measured using three different incubation methods. For experimental design see 2.3. Production expressed as percentage.

	<u>Expt A</u>	<u>Expt B</u>
Method <u>a</u> (bottles)		
Upright	<u>100%</u>	163%
Inclined		<u>100%</u>
Method <u>b</u> (cups)	34%	54%
Method <u>c</u> (sediment cores in tubes)	21%	32%

sediment. Based on the difference in the assimilation of ^{14}C in the inclined bottles of method a and in the cups (method b), the conversion factor 0.6 is used as a compromise correction factor when calculating annual primary production.

Annual microbenthic primary production, calculated on the area under the curves showed year-to-year variation: c. 31, 55 and 71 $\text{g C m}^{-2} \text{ year}^{-1}$ at station 1 for the years 1976, 1977 and 1980 respectively, and 112 and 106 $\text{g C m}^{-2} \text{ year}^{-1}$ at station 2 for the years 1977 and 1980 respectively. Values for winter months, which were not measured each year, were taken from 1976. For 1978 the production for the first half year was calculated as being 15 g C m^{-2} at station 1 and 61 $\text{g C m}^{-2} \text{ year}^{-1}$ at station 2. If the correction factor 0.6 is applied the primary production at station 1 would be 19, 33 and 43 g C m^{-2} per annum and 67 and 64 g C m^{-2} per annum at station 2.

Dark fixation values showed the same general trend in variation as for chlorophyll a and ^{14}C assimilation in light (Figs 4 and 5) with higher values found at station 2 than at station 1 ($\bar{x}$ 3.2 $\text{mg C m}^{-2} \text{ h}^{-1}$ as against 1.6 $\text{mg C m}^{-2} \text{ h}^{-1}$). Dark fixation accounted for c. 10-12% of the total amount of ^{14}C fixed in light, (Appendix 1).

The photosynthetic rate (assimilation number) varied between 0.11 and 1.95 mg C mg chl $a^{-1} h^{-1}$ (Figs 4 and 5, Appendix 1). In general the values were high during summer and low during winter and somewhat higher for station 2.

3.6. Correlation between variables

3.6.1. Approach

Three types of correlation and regression analyses were used to describe relationships between variables (for variables measured and abbreviations see Table 1):

(a) Simple correlations were calculated between all variables measured. Parametric and nonparametric tests gave essentially the same results (Appendices 2 to 3). Since nonparametric tests are often recommended for biological data sets (e.g. Bölter et al. 1980, 1981), diagrams based on Spearman rank correlations are used to give a general view of the relationships (Figs 7 and 8). However, when a better correlation was achieved with a parametric test, this is shown in the diagram and marked with P.

(b) Bivariate scatter plots with simple predictive regression were used to study the relationships between variables in greater detail. Each biotic variable (Chl, Pheo, ProInc, ProDay, DarkFx and AssNo) was plotted against each other variable. Production variables were transformed to logarithmic values. Only scatter plots giving relevant information are shown (Figs 9-12).

(c) Selected variables were used in multiple stepwise regression analyses (Tables 7 to 11) with Chl, ProDay and AssNo as dependent variables. Stations were usually treated together as one data set when AssNo was the dependent variable. The following data sets were used:

(1) Data (means) from the whole investigation (Periods I + II) were analysed to detect general trends of relationships.

(2) Data set (1) was divided into four: values for January-March, April-June, July-September and October-December in accordance with trend of variation in chlorophyll a content and primary production.

(3) Data set (1) was divided according to years: (A) 1976 plus autumn values from 1975, (B) 1977 plus spring values from 1978 and (C) 1980, March-November.

(4) Since 11 extra variables were measured during Period II (1980 = 9 sampling occasions) a separate data set was prepared using values for all parallel samples. Data set (4) was divided into two groups (one for spring and one for the rest of the year), on the indications of the scatter plots. However, since there were only a few observations (15 and 19 for the two groups respectively) the levels of significance were low.

3.6.2. Simple correlations

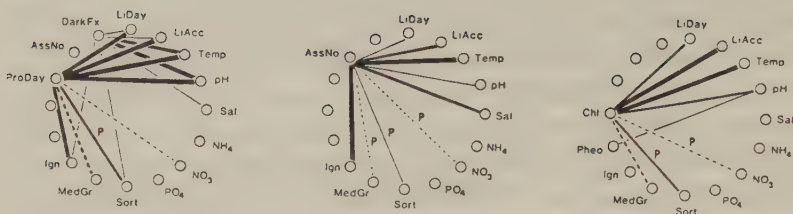
Fig. 7 illustrates simple correlations between 4 biotic (dependent) and 11 abiotic variables. The strongest correlations were found by using the 0.001 level of significance (calculated by t test) in addition to the 0.01 and 0.05 levels.

Daily primary production (ProDay) was strongly positively correlated with light, temperature and pH at both stations, and also with loss on ignition at station 1. ProInc showed similar correlations and only ProDay will be considered below (Fig. 7, Appendices 2-3). The high correlation with pH is due to an increase in pH with increasing photosynthetic activity.

Scatter plots show that ProDay is better correlated with temperature than with light at both stations (Figs 9 and 10). The relationship between production and temperature is logarithmic, corresponding to a 15-17% increase per degree Celsius. When years are treated separately (Data set 3) slightly different regressions are obtained because of varying levels of production (Fig. 9). This also partly accounts for the relation between production and light (Fig. 10).

When Data set (1) is divided into four (=Data set 2), temperature seems to be more limiting at station 1 from January to June and light more limiting from October to December (Table 6). From July to September other factors instead control the microphytobenthic production. At station 2, however,

St. 1



St. 2

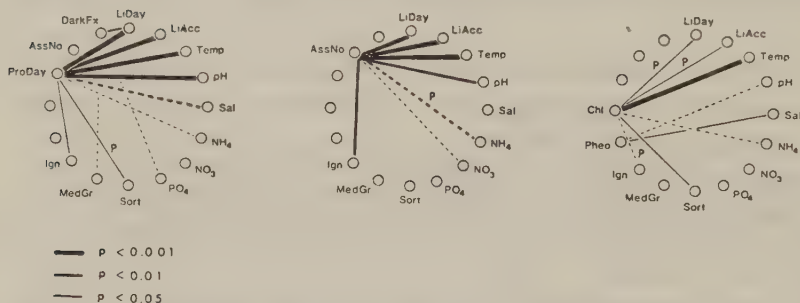


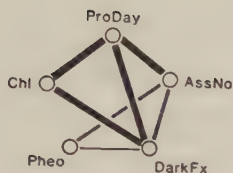
Fig. 7. Spearman rank correlations between 4 biotic variables (ProDay, AssNo, Chl and Pheo) and 11 environmental variables at stations 1 and 2. Parametric correlations are labelled P. Broken lines indicate negative correlations.

temperature seemed to be the major controlling factor even during summer. These relationships must be interpreted with caution since the number of observations was low and the subdivision of Data set (1) arbitrary.

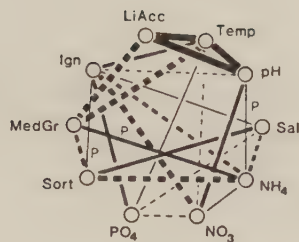
Significant negative correlations were found between daily primary production and NO_3 and between production and median grain size (MedGr) at station 1, and between production and NH_4 and between production and salinity at station 2 (Fig. 7).

Assimilation number (AssNo) was correlated with the abiotic variables in the same way as daily primary production, but showed weaker correlation with light at station 1. When plotted against daily light values (LiDay) the assimilation numbers

St. 1

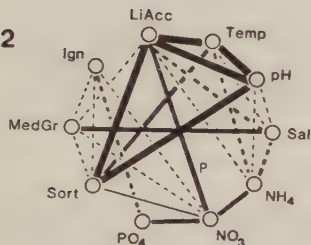


Biotic variables



Abiotic variables

St. 2



Abiotic variables



Biotic variables

— $p < 0.001$ — $p < 0.01$ — $p < 0.05$

Fig. 8. Spearman rank correlations between biotic and abiotic variables. Negative correlations are indicated by broken lines. Parametric correlations are labelled P.

fall into two groups (especially at station 1) indicated by two regression lines: samples from July to March (A) and April to June (B) (Fig. 12). Better correlation between assimilation number and temperature was found if temperature values below 2°C and above 20°C were excluded (Fig. 12).

Chlorophyll a values showed strong positive correlation ($P < 0.001$) with both light and temperature at station 1, but at station 2 with temperature only. Significant though weak negative correlations existed between chlorophyll a content and inorganic nitrogen (NO_3 and NH_4). Few and weak correlations were found between pheopigment and abiotic variables (Fig. 7).

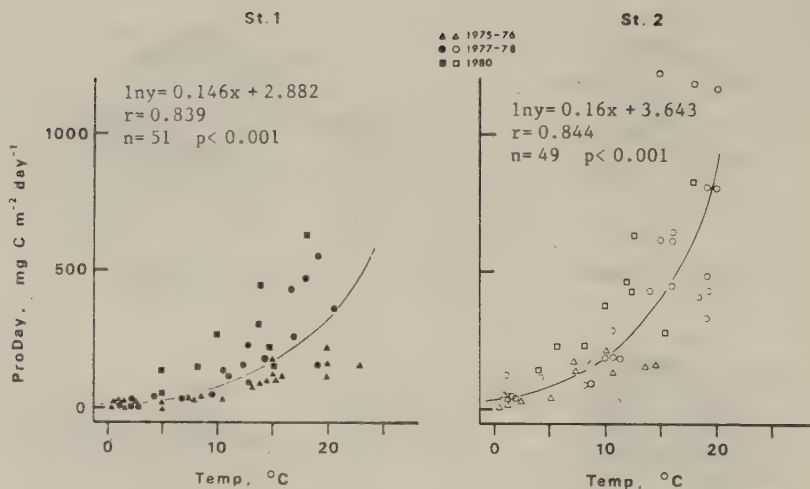


Fig. 9. Relation between daily primary production (ProDay) and temperature at stations 1 and 2.

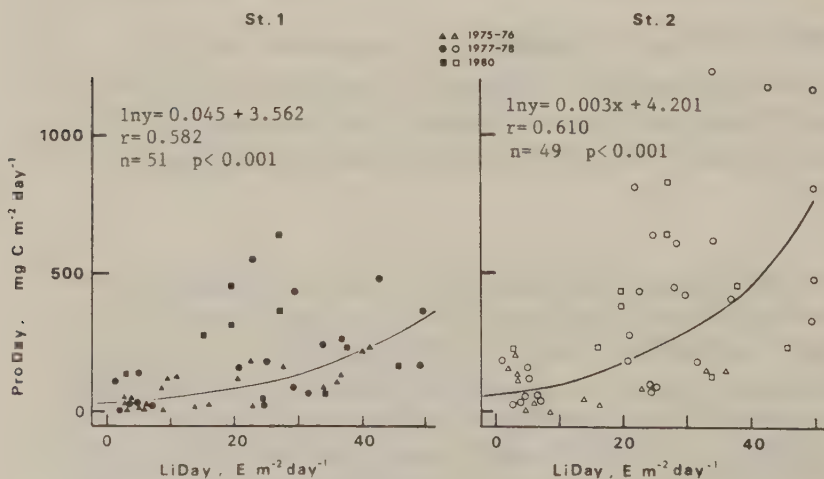


Fig. 10. Relation between daily primary production (ProDay) and daily solar radiation, PAR, (LiDay) at stations 1 and 2.

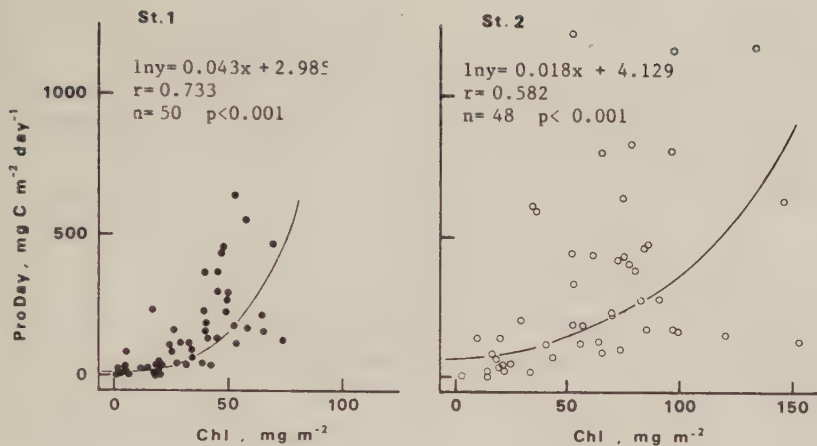


Fig. 11. Relation between daily production (ProDay) and chlorophyll a content (Chl) at stations 1 and 2.

Highly significant correlations existed among the biotic variables ProDay, Chl, AssNo and DarkFx (Fig. 8). Correlation between chlorophyll a content and daily primary production was good but was weakened somewhat by the scattered values at station 2 (Fig. 11). No significant correlation was found between pheopigment and other biotic variables at station 1, but at station 2 negative correlation between pheopigment and chlorophyll a content was observed.

A complicate network of correlations was found between abiotic variables (Fig. 8). Some of these are probably incidental. As expected the strongest positive correlations existed in the triangle formed by accumulated light (LiAcc), temperature and pH (Fig. 8). At station 1 some strong negative correlations were found between sediment characteristics and nutrients. The strong negative correlation between median grain size and temperature at station 1 reflects the calmer conditions of summer. At station 2 the same situation is demonstrated by the strong positive correlation with sorting coefficient. The highly significant negative correlations ($P < 0.001$) found at station 1 were not found at station 2 (Fig. 8).

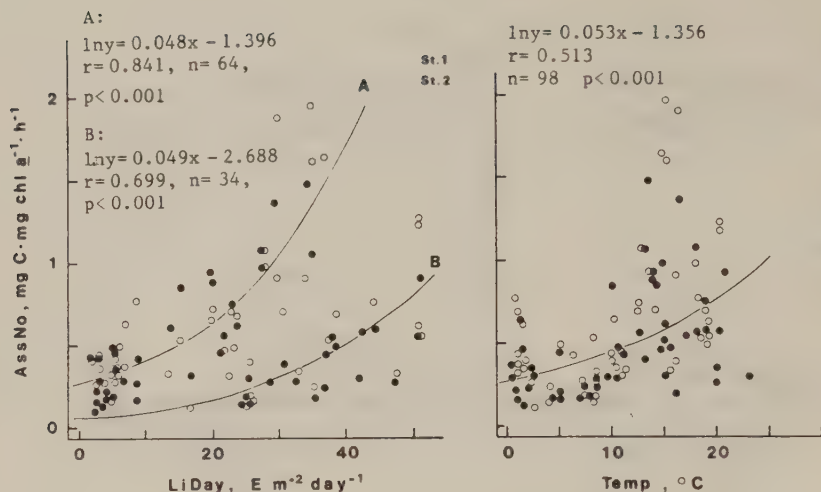


Fig. 12. Relation between photosynthetic rate (AssNo) and daily solar radiation (LiDay) and temperature (Temp) at stations 1 and 2. The separate regression lines (A = July to March, B = April to June) shown for solar radiation. Regression calculations based on both stations together.

Table 6. Correlation between daily primary production (dependent variable), chlorophyll a content, temperature and light (LiDay) for subdivisions of Data set (1). Correlation coefficients $\times 1000$.

Subdivisions of Data set (1)	Station 1				Station 2			
	Chl	Temp	LiDay	% of variation explained	Chl	Temp	LiDay	% of variation explained
Jan - March n = 10	NS	592 ^c	NS	35.0	718 ^c	NS	NS	52.8
Apr - June n = 14 and 17	NS	770 ^b	572 ^c	60.9	NS	727 ^b	543 ^c	53.4
July - Sept n = 15 and 12	NS	NS	NS		NS	696 ^c	526 ^c	51.0
Oct - Dec n = 11 and 10	679 ^c	NS	700 ^c	62.0	NS	NS	NS	

^b $p < 0.01$, ^c $p < 0.05$, NS = not significant

3.6.3. Multiple correlations

The multiple stepwise regression analysis extracted temperature as the most important controlling environmental factor. When temperature was entered into the equations the significance of the other variables usually dropped. Temperature alone explained more than 70% of the variation of ProDay for the whole investigation (Data set 1) at both stations (Tables 7A and 8A). Although it is the most important controlling factor, temperature explained only 46% and 14% of the chlorophyll a variation for stations 1 and 2 respectively (Tables 9A and 10A) and 30% of the variation in assimilation number (Table 11A).

The variables measured throughout the investigation explained at most 85% of the variation (ProDay at station 1, Table 7A). The extra environmental variables measured during Period II (1980 = Data set 4) added between 3 and 24% to the explained variation (Tables 7 to 11B). For daily production one nutrient variable (NO_3) and one sediment variable (sorting coefficient = Sort or proportion of sediment particles $<100\text{ }\mu\text{m}$ = GrA) were entered (Tables 7B and 8B) adding 3.1 and 23.5% for the two stations respectively. When chlorophyll a was the dependent variable only the small particle proportion (GrA) was entered for station 1 and no extra variables at all for station 2 (Tables 9B and 10B). Five extra variables were entered as controlling environmental factors for assimilation number and explained 19% of the variation. All nutrient variables were included, but only the correlation with NO_3 remained unchanged through all steps (Table 11B).

The abiotic variables explained more of the variation in the biotic variables at station 1 than at station 2 (see e.g. Tables 9 and 10). This indicated that the habitat at station 2 is more complex and that there are additional controlling factors there.

Table 7. Multiple stepwise correlation between daily primary production (dependent variable), chlorophyll a content and selected environmental variables at station 1 for the whole investigation (A) and for Period II i.e. 1980 only (B).

Step	Entered variable	% of variation explained	Partial correlation of variables not entered into equation (x 1000)											
			Temp	Chl	LiDay	LiAcc	pH	Sal	NH ₄	NO ₃	PO ₄	GrA	MedGr	Sort
A	0		864 ^a	802 ^a	596 ^a	623 ^a	483 ^a	NS						
1	Temp	0.864		562 ^a	NS	NS	NS	302 ^C						
2	Chl	0.909			NS	NS	NS	363 ^C						
3	Sal	0.922			NS	NS	NS							
			Total 84.9											
B	0		878 ^a	566 ^b	NS		-584 ^b	NS	NS	-438 ^C	NS	NS	-736 ^a	530 ^b
1	Temp	0.878		NS	-755 ^a		-678 ^a	636 ^a	NS	NS	-474 ^C	NS	NS	NS
2	LiDay	0.949					NS	565 ^b	NS	NS	-420 ^C	NS	NS	NS
3	Sal	0.966					NS		NS	502 ^C	NS	-426 ^C	NS	NS
4	NO ₃	0.975					432 ^C		413 ^C		NS	-488 ^C	NS	-518 ^b
5	Sort	0.981					NS		NS		NS	NS	NS	
			Total 96.4											

^a $p < 0.001$, ^b $p < 0.01$, ^C $p < 0.05$, NS not significant

Minimum F to enter value 4.00

Number of observations A: 48 B: 26

Table 8. Multiple stepwise correlation between daily primary production (dependent variable), chlorophyll a content and selected environmental variables at station 2 for the whole investigation (A) and for Period II i.e. 1980 only (B).

Ent red variable				Partial correlaton of variables not entered into equation (x 1000)											
Step	red	r	% of variation explained	Temp	Chl	LiDay	LiACC	pH	Sal	NH ₄	NO ₃	PO ₄	GrA	MedGr	Sort
A	0			844 ^a	582 ^a	598 ^a	639 ^a	523 ^a	NS						
1	Temp	0.844	71.2	-	405 ^b	NS	NS	NS	NS						
2	Chl	0.871	4.7	-	-	NS	NS	NS	NS						
	Total		75.9												
B	0			768 ^a	511 ^b	NS		NS	NS	-533 ^b	NS	NS	422 ^c	NS	412 ^c
1	Temp	0.768	59.1	-	NS	NS	NS	NS	NS	NS	-624 ^b	420 ^c	500 ^c	NS	NS
2	NO ₃	0.866	15.9	-	NS	NS	NS	NS	-391 ^c	398 ^c	-	NS	550 ^b	NS	NS
3	GrA	0.908	7.6	-	NS	NS	NS	NS	NS	NS	-	NS	-	NS	NS
	Total		82.6												

^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$, NS not significant

Minimum F to enter value 4.00

Number of observations A: 49 B: 25

Table 9. Multiple stepwise correlation between chlorophyll a content (dependent variable) and selected environmental variables at station 1 for the whole investigation (A) and for Period II i.e. 1980 only (B).

Step	Entered variable	% of variation r explained	Partial correlation of variables not entered into equation (x 1000)										
			<u>Temp</u>	<u>LiDay</u>	<u>LiAcc</u>	<u>pH</u>	<u>Sal</u>	<u>NH₄</u>	<u>NO₃</u>	<u>PO₄</u>	<u>GrA</u>	<u>MedGr</u>	<u>Sort</u>
A	0		677 ^a	489 ^a	517 ^a	406 ^b	NS						
	1	Temp 0.667	45.9	-	NS	NS	NS						
B	0		634 ^a	NS	480 ^b	-389 ^c	NS	NS	-358 ^c	NS	500 ^b	-546 ^b	502 ^b
	1	Temp 0.634	40.2	-	NS	NS	-352 ^c	NS	NS	NS	507 ^b	NS	NS
	2	GrA 0.745	15.4	-	NS	NS	NS	NS	NS	NS	-	NS	NS
		Total	55.6										

^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$, NS not significant

Minimum F to enter value 4.00

Number of observations A: 60 B: 33

Table 10. Multiple stepwise correlation between chlorophyll a content (dependent variable) and selected environmental variables at station 2 for the whole investigation (A) and for Period II i.e. 1980 only (B):

Step	Entered variable	% of variation explained	Partial correlation of variables not entered into equation (x 1000)										
			Temp	LiDay	LiAcc	pH	Sal	NH ₄	NO ₃	PO ₄	GrA	MedGr	Sort
A	0												
	1	Temp	0.376			14.1							
B	0												
	1	LiAcc	0.444			19.7							

a $p < 0.001$, b $p < 0.01$, c $p < 0.05$, NS not significant

Minimum F to enter value 4.000

Number of observations A:68 B:32

Table 11. Multiple stepwise correlation between assimilation number (dependent variable), chlorophyll a content and selected environmental variables for the whole investigation (A) and for Period II i.e. 1980 only (B). Both stations treated together.

Step	Entered variable	r	% of variation explained	Partial correlation of the variables not entered into equation (x 1000)											
				Temp	Chl	LiDay	LiAcc	pH	Sal	NH ₄	NO ₃	PO ₄	GrC	MedGr	Sort
A				558 ^a	NS	425 ^a	458 ^a	376 ^a	NS						
1	Temp	0.558	31.1	-	-257 ^C	NS	NS	NS	237 ^C						
2	Chl	0.597	4.5	-	-	NS	NS	NS	-288 ^b						
3	Sal	0.640	5.4	-	-	NS	NS	NS	-						
Total				41.0											
B				642 ^a	NS	NS	NS	-342 ^C	NS	-420 ^b	-458 ^a	NS	NS	NS	NS
1	Temp	0.642	41.3	-	NS	-736 ^a	-650 ^a	-506 ^a	295 ^C	NS	-545 ^a	-430 ^b	NS	NS	NS
2	LiDay	0.855	31.8	-	NS	-	NS	301 ^C	NS	NS	-406 ^b	-527 ^a	NS	NS	NS
3	PO ₄	0.898	7.5	-	NS	-	-311 ^C	NS	NS	295 ^C	-442 ^b	-	NS	NS	NS
4	NO ₃	0.918	3.8	-	NS	-	NS	NS	NS	405 ^b	-	-	-296	NS	NS
5	NH ₄	0.932	2.6	-	NS	-	NS	NS	NS	-	-	-	-455 ^b	311 ^C	NS
6	GrC	0.947	2.7	-	NS	-	NS	NS	NS	-	-	-	-	-479 ^a	NS
7	MedGr	0.959	2.4	-	NS	-	NS	NS	NS	-	-	-	-	-	NS
Total				92.1											

^a $p < 0.001$, ^b $p < 0.01$, ^c $p < 0.05$, NS not significant

Minimum F to enter value 4.00

Number of observation A:51 B:96

4. DISCUSSION

4.1. Variation in primary production and chlorophyll a content

The seasonal fluctuation of the microbenthic primary production in the Falsterbo area agrees in general trend with that described for temperate shallow sandy areas, i.e. low winter values and rising values during spring. Regional and spatial differences in the shape of the curve exist, however, such as depressions during summer (e.g. review by van den Hoek et al., 1979; Cadée, 1980; Gargas, 1980). These breaks in the peaks have been accounted for by mechanical disturbance by gales, or biological interference such as by grazing (Cadée and Hegeman, 1974; Taasen and Høisaeter, 1981). A fall in level was also observed in the Falsterbo area in the summer of 1977, but cannot be explained on the basis of the environmental factors measured. The level of microbenthic primary production in different areas is difficult to compare because of the varying techniques used and errors involved. Although I made an attempt to compare different ^{14}C incubation methods it was not possible to exactly copy a method used by other scientists working under different environmental conditions. In addition the corrections for light attenuation vary: no correction at all, correction after comparative experiment (Cadée and Hegeman, 1974; my investigation) or complicated mathematical corrections for laboratory incubations (Gargas and Gargas, 1982). However, the estimated annual production values for the Falsterbo area, except for that at station 1 in 1976, agree with the annual values ($42\text{--}57 \text{ g C m}^{-2}$) from a shallow bay on the Danish coast of Öresund (M. Gargas, 1980; Gargas and Gargas, 1982). The values reported by E. Gargas (1979) for Køge Bay, south of Copenhagen, however, are lower ($\bar{x} 15 \text{ g C m}^{-2} \text{ year}^{-1}$).

The annual microbenthic primary production of the sandy bottoms of shallow waters in Öresund seems to be lower than the mean value ($100 \text{ g C m}^{-2} \text{ year}^{-1}$) estimated for the Wadden Sea (van den Hoek et al., 1979; Cadée, 1980) but higher ($4\text{--}9 \text{ g C m}^{-2} \text{ year}^{-1}$) than for a tidal beach dominated by epipsammic diatoms (Steele and Baird, 1968).

Separate measurements of the filter and sand fractions and microscope examination (Sundbäck, 1983a) indicated the domi-

nance of epipsammic algae. Thus the lower production, compared with that in the Wadden Sea, can be due to the low proportion of the epipellic diatoms typical of tidal areas. However, high proportions of ^{14}C uptake by the sand fraction (70%) have also been reported for tidal flats by for instance Cadée and Hegeman (1974). This indicates that the epipsammic fraction accounts for the greater part of the microbenthic primary production. However, usually only epipellic species are used for ecophysiological experiments of microphytobenthos (cf. Admiraal, 1980), mainly for practical reasons (isolation).

In this three-and-a-half-year investigation somewhat different annual production values were found for the different years. Since the length of sampling intervals probably affects the shape of the production curves, this could not be regarded as certain evidence of actual year-to-year variation. The lower annual production value for 1976 may be bound up with the fact that no measurements were made in August that year, and the smoother curve for 1980 may be a result of the few sampling occasions as compared with Period I. Chlorophyll *a* values, on the other hand, showed no clear year-to-year variation.

Few investigations of microbenthic primary production cover several years. Cadée (1980) reports a range of year-to-year variation from 58 to 177 g C m⁻² year⁻¹ for the Western Wadden Sea during a 12-year period. He concludes that the year-to-year variations in light, temperature or nutrients do not suffice to explain this variation, but good correlation was found between decrease in production and increase in numbers of the gastropod *Hydrobia ulvae*. Variations in grazing effect from year to year cannot be excluded for the Falsterbo stations, but data on the macrofauna exist for 1980 only and no estimation of meiofauna and microfauna was made. Natural densities of the amphipod *Bathyporeia pilosa* have been found to decrease the biomass of microphytobenthos on a sandy bottom in Öresund (Sundbäck and Persson, 1981), but no great numbers were observed at Falsterbo stations at least during 1980. *Hydrobia*, on the other hand, was common and several gastropod species are known to overgraze the microphytobenthos (Fenchel and Kofoed,

1976; Pace et al., 1979). The number of *Hydrobia* seen in 1980, however, was not high enough to suggest heavy grazing.

The overall higher chlorophyll a and production values for station 2 indicate that some factor makes this locality more favourable for microphytobenthic growth. Differences in water depth, salinity and temperature are unlikely to be the cause. The values for nutrients in the interstitial water measured during Period II show no significant differences, but are on the other hand mean values for the upper 4 cm, and thus perhaps do not reflect the nutrient situation in the thin photic zone of the sediment. Some differences in numbers of macrofauna existed, which could result in different levels of grazing and bioturbation, but this influence is difficult to estimate. On tidal flats spatial differences in the amount of benthic microflora present has been found to relate to tidal level in combination with different emersion time and exposure to wave action (Dijkema, 1975; Admiraal and Peletier, 1980; van Es, 1982). The tidal effect is negligible (<10 cm) in the area, but the water level may fluctuate irregularly according to the wind. One clear and demonstrated difference is the degree of exposure to water movement resulting in varying sediment structure (Table 3). The differences in grain size creates differences in the amount of space available for microflora and also affects the penetration of light into the sediment (Haardt and Nielsen, 1980). The slight variation in the colour of the sediment may also affect light attenuation.

Exposure to water movement has been found to control the microphytobenthic biomass by restricting the biomass of motile and loosely attached microalgae (Grøntved, 1960; Colijn and Dijkema, 1981; van den Hoek et al., 1979; Sundbäck, 1983b). Variation in degree of exposure is here reflected in the dominance of different life forms of epipsammic diatoms at the two sampling sites (Sundbäck, 1983a). The diatom flora at station 1 was dominated by flat species attached to the cavities of sand grains (=an *Achnanthes hauckiana*-association). At station 2 protruding species were co-dominants (=a *Fragilaria pinnata*-*Opephora martyi*-association).

4.2. Controlling factors

Of all the abiotic variables considered in this investigation only light and temperature can with any degree of certainty be expected to influence microbenthic primary production. The light factor often lost its significance when temperature was entered into the multiple regression equations. The significant correlations obtained for other abiotic variables such as nutrients and sediment characteristics should be interpreted with caution.

All the correlation analyses show that temperature is the main controlling factor for microphytobenthic growth. The greatest increase of production and biomass (chl a) occurred during the period April to June, i.e. when temperature rises markedly. During the late autumn light, however, seemed to limit production at station 1 but not at station 2, indicating that the microbenthic flora at station 2 may be adapted to lower light intensities due to finer particles, darker sand and more organic matter in the sediment. During summer factors other than temperature and light apparently control the growth at station 1. The significant correlation of primary production with temperature during summer as well at station 2 may be the result of greater variation in temperature (CV=28%) here than at station 1 (CV=15%) during this period.

It has been suggested that biological activity such as grazing (van Es, 1982; Cadée and Hegeman, 1974; Taasen and Høisaeter, 1981) and bioturbation (Joint, 1978) influences the biomass of benthic microflora. In addition, gales have been found to remove the microflora from the sediment surface (Cadée and Hegeman, 1974; Grøntved, 1960). The effects of these factors are, however, difficult to assess in the type of in situ investigation reported here.

Temperature has been found to be the most important explanatory factor in many investigations (Grøntved, 1960; Hargrave, 1969; Gargas, 1970; Gargas and Gargas, 1982). Rasmussen et al., (1983) found an almost linear relationship between temperature and production between 5 and 20°C corresponding to an increase of 5-9% per degree. Colijn and van

Buurt (1975) found an increase of 10% between 4 and 20°C and Admiraal (1977a) and Admiraal and Peletier (1980) demonstrated the close relationship between growth rate and temperature for single diatom species both in laboratory and field incubations. My in situ measurements demonstrate a logarithmic relationship with an even higher increase in production per degree (15-17%) for the whole range of in situ temperatures.

On the whole the role of light appears to be subordinate in controlling the growth of the microphytobenthos in the Falsterbo area. Many investigations have shown that microphytobenthos maintains a high photosynthetic capacity over a wide range of light intensities (Taylor, 1964; Hunding, 1971; Colijn and van Buurt, 1975; Rasmussen et al., 1983). Gargas and Gargas (1982) demonstrated that there was very little difference in photosynthetic capacity for algae from different depths of sediment. If 10,000 lux (c. 0.5 E m⁻² h⁻¹ PAR) is used as a general lower limit for light-saturated photosynthesis (Hunding, 1971; Rasmussen et al., 1983) and corrected for light attenuation in water (factor 0.6 according to Table 2), photosynthesis as observed here would have been light-limited at the sediment surface from late October to early March. However, the level of light saturation varies with season and temperature (Colijn and van Buurt, 1975; Rasmussen et al., 1983).

The microflora was dominated by small diatoms attached to sand grains (Sundbäck, 1983a). Hickman and Round (1970) concluded that "light does not seem to be so important for the maintenance of a high epipsammic standing crop as for epipelon". If this is true the microphytobenthos at both stations was probably above saturation point for light most of the time.

Admiraal (1977a) and Admiraal and Peletier (1980) have shown that species-specific differences in capacity to grow under suboptimal light conditions exist. The weaker correlation of primary production with light for station 2 during the autumn could thus also be a result of the difference in species composition revealed by the diatom analysis (Sundbäck, 1983a).

The use of chlorophyll a content as an estimate of micro-phytobenthic biomass is often criticized because of its poor correlation with organic carbon (cf. de Jonge, 1980) and also because it is not possible to separate different algal groups (cf. Taasen and Høisaeter, 1981). However, the fairly good correlation between primary production and chlorophyll a content I found indicates the usefulness of the chlorophyll a measurements. The weaker correlation at station 2 is probably an effect of greater patchiness (chl a and production not measured from the same sample). Since station 1 is more exposed to the movements of water the sediment is more homogeneous and the patchiness effect is absent (cf. Amspoker, 1977).

The photosynthetic rate expressed as carbon uptake per mg chlorophyll a, here referred to as assimilation number (AssNo), varied between 0.1 and 1.95. Values of photosynthetic rate obtained depend on the incubation method and the depth of chlorophyll a samples. My values (0.1-1.95), however, are well within the range reported by others (Hickman and Round, 1970; Colijn, 1978; Darley et al., 1981; Gargas and Gargas, 1982; Rasmussen et al., 1983).

At first sight assimilation number seems to be better correlated with temperature (at least between 2 and 20°C; Fig. 12) than with light. Gargas and Gargas (1982) found that neither P_b nor $P_{b,m}$ was correlated with light. However, a closer examination revealed that there was one significant regression for summer, autumn and winter (July to March), a period when correlation between production and temperature was weak, and one for April to June, the period when temperature was clearly the major controlling factor. This means that during spring a large biomass may already exist owing to an increase in accumulated light, but that low temperature limits the primary production rate. The grouping of the samples in the scatter plot can scarcely be a coincidence.

The strong positive correlations between primary production and pH was expected, since pH increases as a result of photosynthesis. The significant correlations obtained for salinity are probably accidental or indirect. The small variations

observed are unlikely to affect the production rate if the euryhaline character of brackish-water diatoms is taken into consideration (Admiraal, 1977b). However, high salinities due to evaporation at extreme low water (which may at times occur despite the absence of tides in the area) may inhibit photosynthesis (Rasmussen et al., 1983).

The sandy habitat of the microphytobenthos is usually considered as being rich in nutrients (e.g. van den Hoek, 1979). All correlations between inorganic nutrients and microphytobenthic variables were negative, and would thus, at least theoretically, reflect exchange mechanisms. However, no certain conclusions can be drawn since the concentrations of inorganic phosphorus and nitrogen were high and in addition were measured from the uppermost 4 cm. Recently, however, many investigations have demonstrated that the microbenthic algae may be nutrient-limited (Blackburn and Henriksen, 1979; Henriksen et al., 1980; Darley et al., 1981), and nutrient enrichment experiments with sediment from the Falsterbo area showed in fact that the microphytobenthos was stimulated by the addition of nitrogen (Granéli and Sundbäck, 1983).

Spatial differences in the microphytobenthos have been found to be related to degree of exposure to wave action and hence to sediment structure (Colijn and Dijkema, 1981; Colijn and Nienhuis, 1978; Cadée and Hegeman, 1977; Riznyk and Phinney, 1972). In this investigation the change in sediment structure has, however, been considered in relation to time. There are more and stronger correlations between biotic variables and sediment characteristics at station 1, because the sediment structure alters seasonally more than at station 2. The significant correlations with sorting coefficient and with median grain size both reflect the calmer weather prevailing during summer, and may not be causal. Obviously correlations with sediment characteristics are more informative in describing spatial differences (see Sundbäck, 1983b).

The many correlations found between the abiotic variables (Fig. 8), accidental, indirect or causal, demonstrate the complicated nature of the habitat. However, the variables I have used represent only a small proportion of variables that

could have been included in the statistical analysis (cf. Börlter et al., 1981). Variables that may have increased the percentage of explained variation but that were not or could not be included in the computations are periods of strong wind, turbidity of overlying water and variations in water level.

In conclusion, the variation trends found in this investigation agree with those previously demonstrated for sandy bottoms of other shallow waters. The bottoms, however, differed from those of tidal areas in lacking a well-developed epipelagic flora though this may not be characteristic of the whole of Öresund. The investigation also demonstrates the difficulty of disclosing controlling factors by in situ sampling only and it is necessary to supplement this approach with experiments.

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Appendix 1. Variables measured: Number of observations (n), mean ($\bar{x}$), range, and coefficient of variation (CV,%). For abbreviations see Table 1.

	n	$\bar{x}$	range	CV,%	n	$\bar{x}$	range	CV,%
Chl	65	35.0	2.4 - 83.6	56.6	68	62.4	1.0 - 153.7	55.4
Pheo	65	12.7	0.1 - 53.1	83.5	68	15.9	0.1 - 82.7	94.2
ProInc	51	68.2	5.9 - 235.3	88.0	49	137.0	3.1 - 470.4	83.4
ProDay	51	154.8	8 - 635	98.3	49	334.5	4 - 1242	95.1
DarkFx	50	1.6	0.1 - 9.8	127.4	49	3.2	0.2 - 11.7	87.9
AssNo	50	0.52	0.11 - 1.49	77.0	49	0.63	0.13 - 1.95	70.3
Temp	66	11.9	0.5 - 23.0	55.9	68	11.8	0.5 - 29.5	56.9
LiDay	66	23.1	1.3 - 51.7	66.4	68	21.5	1.3 - 50.8	71.0
LiInc	51	9.3	0.3 - 20.3	63.8	49	9.3	0.3 - 20.3	64.7
LiAcc	66	205.8	17.3 - 460.0	62.9	68	197.7	17.3 - 460.0	66.1
pH	65		7.1 - 9.5		49		7.0 - 9.2	
Sal	61	8.4	7.2 - 11.4	9.8	49	9.4	7.2 - 15.4	14.9
Ign	8	0.20	0.15 - 0.23	14.9	8	0.53	0.32 - 0.9	33.8
NH ₄	9	26.5	14.0 - 44.8	39.7	9	32.9	7.0 - 181.4	136.5
NO ₃	9	4.6	1.4 - 6.8	45.1	9	4.6	1.4 - 8.4	53.0
PO ₄	9	10.5	4.7 - 17.5	42.7	9	7.3	3.6 - 12.5	45.4
GRA	8	0.19	0.1 - 0.26	34.1	8	2.58	1.71 - 3.34	21.7
GRB	8	0.65	0.27 - 1.05	43.3	8	6.00	4.44 - 6.83	15.3
GrC	8	40.5	0.42 - 57.8	43.4	8	59.0	52.3 - 60.5	2.2
GrD	8	48.0	37.5 - 62.8	17.0	8	28.6	25.9 - 31.9	6.8
GrE	8	11.8	2.6 - 53.8	157.9	8	3.8	3.4 - 4.7	13.0
MedGr	8	0.255	0.235 - 0.280	6.1	8	0.210	0.200 - 0.225	2.3
Sort	8	0.45	0.38 - 0.47	6.1	8	0.48	0.44 - 0.51	4.4

Appendix 3a. Correlation (parametric) between variables at station 2 from the whole investigation (above line $n = 45--68$) and from period II only (below line $n = 25--32$).

Chl	1000	Pheo	ProInc	ProDay	DarkFx	AssNo	Temp	LiDay	LiInc	LiAcc	pH	Sal	Ign	NO ₃	PO ₄	GrA	MedGr	Sort
Chl	1000																	
Pheo	-539	1000																
ProInc	748	-301	1000															
ProDay	582	-190	960	1000														
DarkFx	475	245	519	493	1000													
AssNo	-181	102	685	666	220	1000												
Temp	455	-282	806	844	532	544	1000											
LiDay	247	-271	634	605	389	480	643	1000										
LiInc	498	-242	521	521	383	342	612	932	1000									
LiAcc	281	-258	640	639	432	498	744	869	848	1000								
pH	186	-299	678	523	206	422	655	536	679	530	1000							
Sal	-197	177	-260	-224	-139	-16	140	-408	-393	-381	-160	1000						
Ign	-440		243	-158	424	-55	-495		-267	-188	-285	1000	NH ₄					
NH ₄	-368		-119	-139	-616	-581	160		50	-309	-309	-224	1000					
NO ₃	63		-256	-121	-491	162	464		470	265	-172	-476	397	1000				
PO ₄	269		-26	222	-114	328	100		216	97	97	-515	544	513	1000			
GrA	78		442	321	396	217	267		343	354	-664	327	164	-75	165	1000	MedGr	
MedGr	-174		-215		-55	-260	-288		-451	-442	917	-215	-118	-332	-74	-713	1000	Sort
Sort	317		412		613	412	916		889	272	-272	-283	101	422	217	539	-514	1000

Appendix 3b. Spearman rank correlation between variables at station 2 from the whole investigation (above line n = 45--68) and from Period II only (below line n = 25--32).

<u>Ch1</u>		<u>Pheo</u>		<u>ProInc</u>		<u>ProDay</u>		<u>DarkFx</u>		<u>AssNo</u>		<u>Temp</u>		<u>LiDay</u>		<u>LiInc</u>		<u>LiAcc</u>		<u>pH</u>		<u>Sal</u>		<u>Ign</u>	
Ch1	1000	Pheo	1000	ProInc	1000	ProDay	1000	DarkFx	1000	AssNo	1000	Temp	1000	LiDay	1000	LiInc	1000	LiAcc	1000	pH	1000	Sal	1000	Ign	1000
Pheo	-297																								
ProInc	662	-484																							
ProDay	580	-303	960																						
DarkFx	361	-174	406	418																					
AssNo	-121	14	605	629	240																				
Temp	475	-361	804	870	411	558																			
LiDay	181	-116	546	583	363	528	697																		
LiInc	254	-122	504	467	321	400	567	910																	
LiAcc	244	-182	622	681	361	572	789	915	820																
pH	202	244	555	564	354	423	690	679	558	650															
Sal	-228	219	-315	-369	-133	-154	-347	-392	-237	384	-348														
Ign	-139		471	409	328	610	36	-523	-554	-98	-264	-238													
NH ₄	-358		-301	-398	-139	-289	-576	-324	-272	-438	-409	-541	114												
NO ₃	-11		-424	-296	-60	-464	107	332	295	241	185	-205	-383	500											
PO ₄	-11		-151	-137	-431	-252	107	90	241	22	-206	-272	-493	232	554										
GrA	-133		207	270	-75	170	24	270	174	168	172	-305	194	200	107	339									
MedGr	-196		-137	-248	-412	-103	-258	-412	-309	-412	-413	570	-337	-155	-412	0	-571								
Sort	375		92	323	98	-6	569	956	936	858	913	-29	-425	-236	443	108	258	-175							

REFERENCES

- Admiraal, W. 1977a: Influence of light and temperature on the growth rate of estuarine benthic diatoms in culture. - *Mar. Biol.* 39: 1-9.
- Admiraal, W. 1977b: Salinity tolerance of benthic estuarine diatoms tested with a rapid polarographic measurement of photosynthesis. - *Mar. Biol.* 39: 11-19.
- Admiraal, W. 1980: Experiments on the ecology of benthic diatoms in the Ems-Dollard estuary. - *Doct. thesis*, BOEDE publ. No. 3. 125 pp.
- Admiraal, W. and Peletier, H. 1980: Influence of seasonal variations of the temperature and light on the growth rate of cultures and natural populations of intertidal diatoms. - *Mar. Ecol. Prog. Ser.* 2: 35-43.
- Amspoker, M.C. 1977: The distribution of intertidal epipsammic diatoms on Scripps Beach, La Jolla, California, USA. - *Bot. Mar.* 20: 227-232.
- Blackburn, T.H. and Henriksen, K. 1979: Regenerering af naeringssalte fra sedimenter. - *Vand* 4: 117-122.
- Bölter, M., Meyer, M. and Probst, B. 1980: A statistical scheme for structural analysis in marine ecosystems. - *Ecol. Model.* 9: 143-151.
- Bölter, M., Meyer-Reil, L.-A., Dawson, R., Liebezeit, G., Wolter, K. and Szwering, H. 1981: Structure analysis of shallow water ecosystems: Interaction of microbiological, chemical and physical characteristics measured in the overlying waters of sandy beach sediments. - *Est. Coast. Shelf Sc.* 13: 579-589.
- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. - *Neth. J. Sea Res.* 8: 260-291.
- Cadée, G.C. and Hegeman, J. 1977: Distribution of primary production of the benthic microflora and accumulation of organic matter on tidal flat area, Balgzand, Dutch Wadden Sea. *Neth. J. Sea Res.* 11: 24-41.
- Cadée, G.C. 1980: Reappraisal of the production and important of organic carbon in the western Wadden Sea. - *Neth. J. Sea res.* 14: 305-322.
- Carlberg, R. (ed.) 1972: New Baltic manual with methods for sampling and analyses of physical, chemical and biological parameters. - ICES, Cooperative Research Report, Ser. A, No. 29. 145 pp.
- Colijn, F. 1978: Primary productivity measurements in the Ems-Dollard estuary during 1975 and 1976. Publications and reports of the project 'Biological Research in the Ems-Dollard estuary'.
- Colijn, F. and van Buurt, G. 1975: Influence of light and temperature on the photosynthetic rate of marine benthic diatoms. - *Mar. Biol.* 31: 209-214.
- Colijn, F. and Koeman, R. 1975: Das Mikrophytobenthos der Watten, Strände und Riffe um den Hohen Knechtsand in der Wesermündung. - *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 26: 53-83.
- Colijn, F. and Nienhuis, H. 1978: The intertidal microphytobenthos of the 'Hohe Weg' shallows in the German Wadden Sea. - *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 29: 149-174.
- Colijn, F. and Dijkema, K.S. 1981: Species distribution of benthic diatoms and distribution of chlorophyll *a* on an intertidal flat in the Dutch Wadden Sea. - *Mar. Ecol.* 4: 9-21.
- Darley, W.M., Montague, C.L., Plumley, F.G., Sage, W.W. and Psalidas, A.T. 1981: Factors limiting edaphic algal biomass and productivity in a Georgia salt marsh. - *J. Phycol.* 17: 122-128.
- Dijkema, K.S. 1975: Verkennend onderzoek naar de invloed van abiotische factoren op de benthische diatomeen in de oostelijke Waddenzee. Publications and reports of the project 'Biological Research in the Ems-Dollard estuary'.

- Dixon, W.J. (ed.) 1981: BMDP statistical software. - 726 pp. Berkeley.
- Es, van, F.B. 1982: Community metabolism of intertidal flats in the Ems-Dollard estuary. - Mar. Biol. 56: 95-108.
- Fenchel, T. and Kofoed, L.H. 1976: Evidence for exploitative inter-specific competition in mud snails (Hydrobiidae). - Oikos 27: 367-376.
- Fenchel, T. and Straarup, B.J. 1971: Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. - Oikos 22: 172-182.
- Gargas, E. 1970: Measurements of primary production, dark fixation and vertical distribution of microbenthic algae in the Öresund. - Ophelia 8: 231-253.
- Gargas, E. (ed.) 1975: A manual for phytoplankton primary production studies in the Baltic. - The Baltic Marine Biologists Publ. No. 2. 88 pp. Copenhagen.
- Gargas, E. 1979: Mikrobenthosalgproduktion. - Vandkvalitetsundersøgelser i Køge Bugt 1977/78. Report No. 15. 98 pp.
- Gargas, M. 1980: Ökofysiologi og artsammanseættning hos mikrobenthiske diatomeer i Nivå bugt. - Thesis, University of Copenhagen, 384 pp. Copenhagen.
- Gargas, M. and Gargas, E. 1982: Growth physiological conditions of marine microalgae in the topmost 10 cm of the sediment. - Vatten 38: 189-198.
- Granéli, E. and Sundbäck, K. 1983: Response of planktonic and microbenthic algal assemblages to nutrient enrichment in shallow coastal waters, SW Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Gray, J.S. 1981: The ecology of marine sediments. - 185 pp. Cambridge.
- Grøntved, J. 1960: On the productivity of microphytobenthos and phytoplankton in some Danish fjords. - Medd. Danm. Fiskeri- og Havunders. 3: 55-92.
- Grøntved, J. 1962: Preliminary report on the productivity of microbenthos and phytoplankton in the Danish Wadden Sea. - Medd. Danm. Fiskeri- og Havunders. 3: 347-372.
- Haardt, H. and Nielsen, G.A. 1980: Attenuation measurements of monochromatic light in marine sediments. - Oecologica Acta 3: 333-338.
- Hargrave, B.T. 1969: Epibenthic algal production and community respiration in the sediments of Marion Lake. - J. Fish. Res. Bd Can. 26: 2003-2026.
- Henriksen, K., Hansen, J.I. and Blackburn, T.H. 1980: The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. - Ophelia, Suppl. 1: 259-256.
- Hickman, M. and Round, F.E. 1970: Primary production and standing crops of epipsammic and epipelagic algae. - Br. phycol. J. 5: 247-255.
- Hoek, van den, C., Admiraal, W., Colijn, F. and de Jonge, V.N. 1979: The role of algae and seagrasses in the ecosystem of the Wadden Sea: A review. - In Wolff, W.J. (ed.) Flora and vegetation of the Wadden Sea: 3/1-206. Rotterdam.
- Hunding, C. 1971: Production of benthic microalgae in the littoral zone of an eutrophic lake. - Oikos 22: 389-397.
- Jansson, A.-M., Kautsky, N., von Oertzen, J.-A., Schramm, W., Sjöstedt, B., von Wachenfeldt, T. and Wallentinus, I. 1982: Structural and functional relationships in a southern Baltic *Fucus* ecosystem. A joint study by the BMB phytobenthos group. BMB Publ. 8. 96 pp. Stockholm.
- Jerlov, N.G. 1974: A simple method for measuring quanta irradiance in the ocean. - Report 24, Inst. of Physical Oceanography, University of Copenhagen.
- Joint, I.R. 1978: Microbial production of an estuarine mudflat. - Estuar. Coast. Mar. Sc. 7: 185-195.
- Jonge, de, V.N. 1980: Fluctuations in the organic carbon to chlorophyll *a* ratios for benthic diatoms populations. - Mar. Ecol. Prog. Ser. 2: 345-353.

- Leach, J.H. 1970: Epibenthic algal production in an intertidal mudflat. - *Limnol. Oceanogr.* 15: 514-521.
- Lorenzen, C.J. 1967: Determination of chlorophyll and pheopigments. Spectrophotometric equations. - *Limnol. Oceanogr.* 12: 343-346.
- Marshall, N., Skauen, D.M., Lampe, H.C. and Oviatt, C.A. 1973: Primary production of benthic microflora. In: A guide to measurement of marine primary production under some special conditions. - *Unesco Monographs on oceanographic methodology* 3: 37-44.
- Pace, M.L., Schimmel, S. and Darley, W.M. 1979: The effect of grazing by a gastropod, *Nassarius obsoletus*, on the benthic microbial community of a salt marsh mudflat. - *Estuar. Coast. Mar. Sci.* 9: 121-134.
- Pamatmat, M.M. 1968: Ecology and metabolism of a benthic community on an intertidal sandflat. - *Int. Rev. ges. Hydrobiol.* 53: 211-298.
- Rasmussen, M.B., Henriksen, K. and Jensen, A. 1983: Possible causes of temporal fluctuations in primary production of the microphytobenthos in the Danish Wadden Sea. - *Mar. Biol.* 73: 109-114.
- Revsbech, N.P., Jørgensen, B.B. and Brix, O. 1981: Primary production of microalgae in sediments measured by oxygen profile, $H^{14}CO_3$ -fixation, and oxygen exchange methods. - *Limnol. Oceanogr.* 26: 717-730.
- Riznyk, R.Z. and Phinney, H.K. 1972: The distribution of intertidal phytosammon in an Oregon estuary. - *Mar. Biol.* 13: 318-324.
- Schindler, D.W. and Holmgren, S.K. 1971: Primary production and phytoplankton in the experimental lakes area, northwestern Ontario, and other lowcarbonate waters, and a liquid scintillation method for determining ^{14}C activity in photosynthesis. - *J. Fish. Bd Canada* 28: 189-201.
- Steele, J.H. and Baird, I.E. 1968: Production ecology of a sandy beach. - *Limnol. Oceanogr.* 13: 14-25.
- Steemann Nielsen, E. 192: Use of radio-active carbon (C^{14}) for measuring organic production in the sea. - *J. Cons. perm. int. Explor. Mer.* 18: 117-140.
- Sundbäck, K. 1983a: The species composition of benthic diatom assemblages on a sandy coast with shallow brackish water, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. 1983b: Spatial variation in chlorophyll *a* content and species composition at diatom assemblages in relation to sediment characteristics in a non-tidal shallow bay, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. and Persson, L.-E. 1981: The effect of microbenthic grazing by an amphipod, *Bathyporeia pilosa* Lindström. - *Kieler Meeresforsch., Sonderh.* 5: 573-575.
- Taasen, J.P. and Høisaeter, T. 1981: The shallow-water soft-bottom benthos in Lindåspollene, Western Norway. 4. Benthic marine diatoms, seasonal density fluctuations. - *Sarsia* 66: 293-316.
- Taylor, W.R. 1964: Light and photosynthesis in intertidal benthic diatoms. - *Helgol. Wiss. Meeresunt.* 10: 29-39.
- Wachenfeldt, von, T. 1975: Marine benthic algae and the environment in the Öresund. Diss. University of Lund, 328 pp. Lund.
- Wachenfeldt, von, T. 1978: Forskningsredogörelse för materialbalansstudier i Öresund, Botanisk-kemisk del. - Report from Department of Marine Botany, Lund.
- Wachenfeldt, von, T. 1980: Sammanställning av närsaltsförhållandena i Öresund. - Department of Marine Botany, Lund. Mimeographed.
- Öresundskommissionen 1980: Öresund. Tillstånd - effekter av närsalter. - 252 pp. Stockholm.

THE SPECIES COMPOSITION OF BENTHIC DIATOM ASSEMBLAGES ON A
SANDY COAST WITH SHALLOW BRACKISH WATER, S ÖRESUND, SWEDEN

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ABSTRACT

The species composition of benthic diatom assemblages on two sandy bottoms was examined at intervals over a period of years to investigate whether the seasonal and spatial variations in microbenthic chlorophyll a content and primary production were related to structural variation. The usefulness of an artificial substrate (glass) for studying sediment-associated diatom assemblages was also tested. The diatom flora of the sediment was dominated by small epipsammic species (cell length $<20\text{ }\mu\text{m}$), which accounted for c. 70--80% of the total cell volume of diatoms at the both sites. The diatom flora of the two sites separated out as two groups: (1) an *Achnanthes hauckiana*-association dominated by adnate species in the cavities of sand grains, and (2) a *Fragilaria pinnata*-*Opephora martyi*-association where protruding species were co-dominants with the adnate species. It is suggested that exposure to water movement restricts the amount of protruding life forms and this is reflected as lower chlorophyll a values. The species composition of diatoms in the sediment was fairly stable all year round and ordination revealed no clear successional trend. Diversity (H') and evenness (J') were high and varied little during the investigation. The lack of seasonal variation together with high diversity supports the hypothesis, presented by others, that the epipsammic flora of sandy sediments consists of life forms well adapted to their habitat (e.g. high tolerance of mixing by water movements and protracted burial).

The diatom flora of the glass substrate formed a separate association dominated by *Cocconeis placentula*. This association was characterized by a clear successional trend, lower and more varying diversity and lower dimensionality than in the sediment flora owing to the invasion of *Cocconeis placentula*. The glass substrate was colonized by epiphytic rather than epipsammic species.

1. INTRODUCTION

Early investigations of littoral microphytobenthic communities were either production measurements made by ecologists (e.g. Pomeroy, 1959; Grøntved, 1960; Pamatmat, 1968) or qualitative distributional studies by taxonomists (Brockmann, 1950; Hustedt, 1939, 1955; Simonsen, 1962; van der Werff, 1960). Later these types of investigation were replaced by quantitative analyses of distribution in relation to environmental factors, often including multivariate statistical analyses (e.g. McIntire and Overton, 1971; McIntire, 1973, 1978).

The high production capacity of microphytobenthos in shallow waters was demonstrated by several investigators in the early 70's (e.g. Gargas, 1970, 1971; Cadée and Hegeman, 1974). Further advances in this field required the conjunction of biomass or production measurements with structural investigations (cf. review by McIntire and Moore, 1977) such as by e.g. Colijn and Nienhuis (1978), Colijn and Dijkema (1981) as well as ecophysiological experiments on isolated single species or mixed populations of benthic diatoms (Admiraal, 1980; Admiraal et al., 1982; Gargas and Gargas, 1982a, b).

My investigation attempts to combine production measurements and determination of the species composition of the benthic microflora, mainly diatoms, of two shallow, sandy substrates in Öresund, which is a brackish strait between the Kattegat and the Baltic Sea. When the investigation was initiated in autumn (October) 1975 the only microphytobenthic studies from Öresund that had been published were those presenting production measurements from the Danish coast by Gargas (1970, 1971). No information on species was available from the area, except for Simonsen's (1962) examinations of some sediment samples from the deeper parts of Öresund.

Initially my qualitative investigation was planned to comprise regular extensive sampling along the whole salinity gradient of Öresund, but this plan was soon abandoned and the structural study was concentrated to the same stations as for measurements of primary production and chlorophyll a in the Falsterbo area (Sundbäck 1983).

Preliminary examinations of fresh sediment collections showed that diatoms were the main constituents of the microphyto-

benthos in the sandy sediment, and the quantitative identifications were restricted to this group.

The aims of the investigation were:

- (1) To investigate whether seasonal and spatial variation of biomass and production were related to species composition
- (2) To compare the species composition of the benthic diatom assemblages from a shallow non-tidal brackish area with that described from other, mainly tidal areas.
- (3) To get information that can serve as a base for further investigations on the effect of low salinity on the morphology of diatom species, and for experiments on the influence of grazing, nutrient loading and pollution. The Falsterbo area does not receive any local discharge and may thus also serve as a 'clean' control area for Öresund and the SW Baltic.
- (4) To test whether an artificial substrate (glass) can be used to investigate the species composition of the benthic diatom assemblages of a sandy sediment.

Ordination and classification were used to reduce dimensionality and extract the main trends of distribution.

2. MATERIAL AND METHODS

2.1. Study area

The investigation was carried out on two sandy bottoms in shallow water on the Falsterbo peninsula, SW Sweden, just in the transition zone between Öresund and the Baltic Sea (Fig. 1). Öresund is one of the three passages leading from the Kattegat to the Baltic and is characterized by horizontal and vertical salinity gradients. The dominating nearshore habitat is as in the Falsterbo area (see Öresundskommissionen, 1980).

A general description of the Falsterbo area is given in Jansson et al. (1982). Hydrography and sediment characteristics have been previously described together with microphytobenthic primary production and chlorophyll a content (Sundbäck, 1983). The main characteristics of the sampling stations are summarized in Table 1.

In brief: Station 1 is situated in the basin at the southern entrance to the Falsterbo canal (Fig. 1) between a

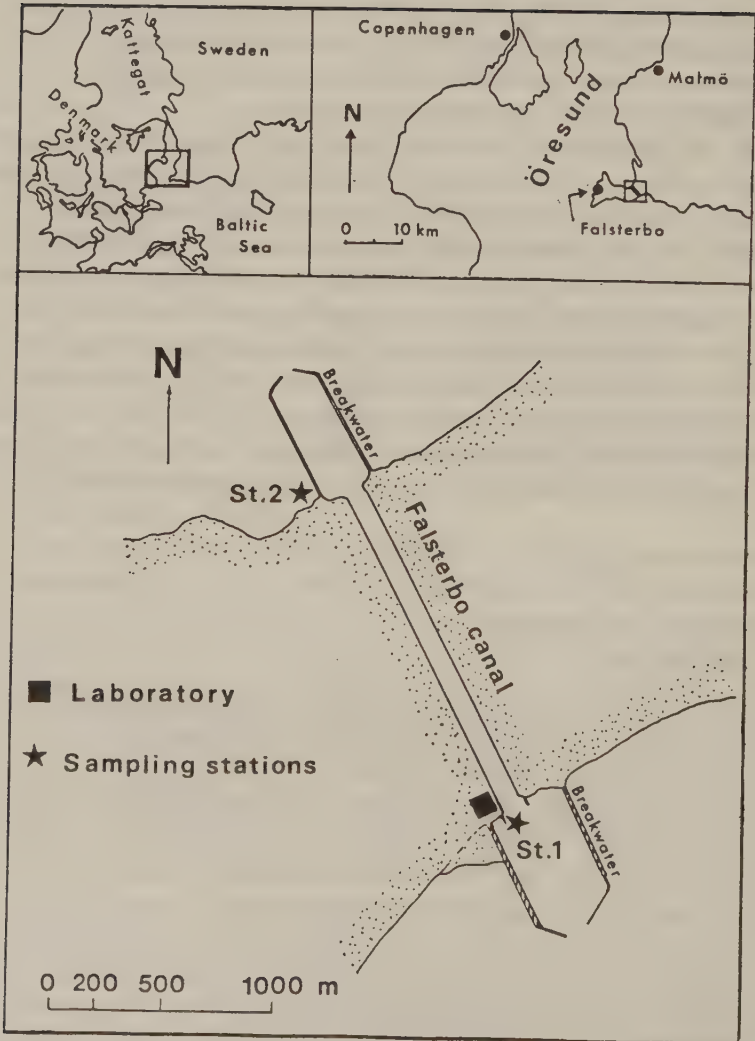


Fig. 1. The two sampling stations on the Falsterbo peninsula.

small stone jetty and a *Fucus vesiculosus* patch. The substrate consists of clean, well-sorted, fine to medium, pale sand, with no macroalgae (Table 1). The site is exposed to the swell from canal traffic.

Station 2 is situated in shallow water outside the break-water immediately west of the northern entrance to the Falsterbo canal, about 2 km from station 1 (Fig. 1). It is thus more sheltered since it is not exposed to the swell from canal traffic and because the waves break a considerable way out. The sediment is a well-sorted, fine, yellowish-grey sand (Table 1) with patches of macroflora, mainly *Ruppia* sp. In late summer a carpet of decaying macroalgae and *Zostera marina* L. accumulates along the shore.

2.2. Sampling

Sediment samples for studying species composition were taken simultaneously with samples for chlorophyll a content and primary production measurements (Sundbäck, 1983). The investigation was carried out during two periods: October 1975 to June 1978 (Period I) and April 1980 to November 1980 (Period II). Samples from 29 occasions (Appendix 1), the majority from 1977 and 1980, are included in the investigation.

The sampling procedures have been previously described in detail (Sundbäck, 1983). Briefly, 2--4 samples of the sediment were taken from the top 0.5 cm. During Period I the area of a sample was 3.14 cm², while during Period II 3 samples of 0.64 cm² were pooled. The samples were transported to the laboratory (Kämpinge Research Station, National Swedish Environment Protection Board) in thermally insulated boxes.

2.3. Counting and identification

Fresh samples were examined under the microscope (x 125 and x 500 magnification) so that the proportions of organisms other than diatoms could be roughly estimated. The samples

Table 1. Some characteristics of the two sampling stations. Number of sampling days (S.d.) mean ($\bar{x}$) and range (in brackets) are shown. From Sundbäck, 1983.

	Station 1			Station 2		
	S.d.	$\bar{x}$	Range	S.d.	$\bar{x}$	Range
Exposure to wind, direction						
Exposed to swell from canal traffic		SSE-E			W-NW	
Depth of water, m	66	yes	0.35 (0-0.6)	49	no	0.30 (0-0.6)
Salinity, ‰	61	8.4	(7.2-11.4)	49	9.4	(7.2-15.4)
Median grain size, mm *	8	0.255	(0.235-0.28)	8	0.21	(0.2-0.255)
Sorting coefficient, σ_g	8	0.45	(0.38-0.47)	8	0.48	(0.44-0.51)
Loss on ignition, % of dry weight *	8	0.2	(0.15-0.23)	8	0.53	(0.32-0.9)
Chlorophyll a , mg m ⁻²	65	35.0	(2.4-83.6)	49	62.4	(1.0-153.7)
Primary production, mg C m ⁻² day ⁻¹	51	154	(8-635)	49	334	(4-1242)
Macrofauna, indiv m ⁻²	7	1,280	(360-3,300)	7	3,900	(2,000-9,500)

* measured only during 1980 (Period I)

were preserved with neutralized formalin (Period I) or Lugol's solution (Period II). Subsamples of the replicate collections were pooled and, if already preserved, washed free of fixation by repeated washing and centrifuging (10 minutes, 3500 rpm). The organic cell content was removed by oxidation using either the H_2O_2 - or KMnO_4 -method. The diatom suspension was cleaned from the oxidation medium by repeated washing and centrifuging. Permanent microscope slides were prepared using Mikrops ($n_d=1.64$) or Naphrax ($n_d=1.74$). Occasionally separate slides were prepared from the sand and suspendible fractions after repeated washing with filtered seawater (Round, 1965; Moss and Round, 1967).

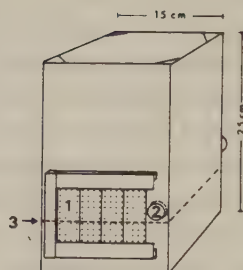
About 350 (Period I) or 500 (Period II) diatom valves were identified and counted along central, linear transects from two replicate slides at x 1250 magnification (Leitz Dialux 20 EB). Only undamaged valves were included in the counts. The identification involved taxonomical problems (see 4.5.).

The abundance for each taxon was expressed as percentage of the total count, i.e. relative abundance (RA, %). Any species not recorded in the transects counted but observed more than once by scanning an additional 1--2 transects, was given the relative abundance value of 0.1.

The diatom species were identified according to Hustedt (1930, 1939, 1930--1966, 1955) and Cleve-Euler (1951--1955) supplemented by works by Hendey (1964), Patrick and Reimer (1966), Pankow (1976), van der Werff and Huls (1957--1974) and articles by Håkansson and Stabell (1977), Lange-Bertalot (1977, 1980), Lange-Bertalot and Ruppel (1980) and Simonsen (1959, 1960).

When the second sampling period (1980) was planned, it was decided to re-count the samples from Period I so that the artifacts due to inconsequent identification could be minimized. In the course of this work it became evident that much of the material from 1976 had disappeared when the department moved to another building in 1978. Thus most of the samples collected during 1976 were omitted from the analyses.

Fig. 2. PVC frame used in colonization experiments. 1 = glass slide, 2 = stopper, 3 = sediment surface



2.4. Colonization of artificial substrates

During Period II (1980) a PVC frame containing glass slides for colonization was placed at station 2 (Fig. 2). The frame was pressed down so that the slides were halfway submerged in the sediment. The slides were immersed for 4(6) and 8 weeks. On two occasions four of the eight slides were found to have disappeared. In all, eight colonization periods could be studied during the period 4 April to 16 November (Table 2). When collected the slides were placed vertically in plastic jars containing filtered seawater and transported to the field laboratory in a thermally insulated box.

Two of four replicate slides were examined and photographed under the light microscope at x 125 and x 500 magnifications to view the type and degree of colonization. A rough estimate of the relative abundance of different types of organisms was made. The organisms on the two remaining replicate slides were scraped off with a scalpel and rinsed into beakers. The duplicate slides were treated separately. The suspension of organisms was concentrated by centrifuging, and permanent slides for diatom counts were prepared after H_2O_2 -oxidation (see 2.3.). About 350 valves per slide were counted.

Table 2. Colonization of glass slides at station 2 during 1980 (Period II).

<u>Period</u>	<u>Length of period in days</u>
(1) 1980-04-06--05-18	42
(2) 04-06--06-01	55
(3) 06-01--06-29	29
(4) 06-29--07-27	28
(5) 06-29--08-24	56
(6) 08-24--09-14	26
(7) 08-24--10-19	60
(8) 10-19--11-16	28

2.5. Numerical analyses

The numerical analyses are based on relative counts of oxidized diatom cells. The errors involved when this method is used due to the exclusion of algae other than diatoms and the inclusion of dead diatom frustules have been pointed out by Round (1971) and Thomas (1979) among others. However, cleaned diatom suspensions were used in this study since the ornamentation of small forms could only be seen after organic cell content had been removed. The influence of the use oxidized diatom cells in this investigation will be treated in 3.3 and 4.4.

Three types of numerical analysis were used, i.e. ordination, classification and calculations of diversity. The ordination program ORDINA (Roskam, 1971) run according to the instructions in Persson (1978), performs a centered non-standardized principal component analysis (PCA) from a matrix of Euclidean distances between samples. The program produces ordination diagrams that illustrate the trend and direction of dissimilarities between the samples or clusters along axes which can be interpreted as gradients of a factor or group of factors. The classification program TABORD (van der Maarel et al., 1978; Persson, 1977) performs an agglomerative clustering of samples based on similarity ratio (SR) and produces a phytosociological table with a diagonal structure. The classification was used to determine

(a) whether the diatom communities could be identified as discrete assemblages representing the two stations and the colonized glass slides and (b) whether clusters indicating clear successional changes in the diatom flora existed.

The following options available in the TABORD program were used: minimum cluster size (2), threshold value (0.2) and constant species (defined as taxonomic units with at least 70% frequency in a cluster). The initial classification required for the program was achieved by grouping adjacent samples according to the ordinations.

The Shannon index H' (Shannon and Weaver, 1949) and the evenness index J' (Pielou, 1966), calculated according to the formulae in Liljelund (1977), were used to describe diversity and evenness respectively. These indices were calculated from the same data matrix as the classification and ordination and were thus based on relative abundance, not individuals.

$$H' = - \sum_{i=1}^S \frac{n_i}{N} \log_2 \frac{n_i}{N}$$

S = number of taxa in the sample

N = number of individuals in the sample

n_i = number of individuals of i th taxon

$$J' = \frac{H'}{H' \max} \quad \text{where } H' \max = \log_2 S$$

Before submitting the species composition data to the computer it was edited as follows:

Rare taxa were excluded (21 out of 145 taxa in the species list; Appendix 2). Subspecific taxa that were difficult to delimit and that were co-occurrent were combined to form a single taxonomic unit (e.g. *Cocconeis placentula* et var., *Navicula cryptocephala* et var., *Navicula salinarum* et var.). Uncertain or unidentified but distinct taxa were given numbers (see Appendix 2) (15 out of 124 taxa included in the computations).

2.6. Cell volume

Since cells are of varying size cell counts may give a false picture of the proportions of individual species in the total biomass. Therefore I made a rough estimate of the contribution of different species based on cell volume, although this was not initially included in the investigation. The rough mean cell volume for a species was based on 50--75 specimens from four randomly chosen slides from each station and the colonized glass slides. The cell volume was calculated according to the recommendations in Edler (1979), with certain modifications of the formulae because of cell shape. Only constant species were considered (see 2.5.).

3. RESULTS

3.1. Characteristics of the microflora based on fresh sediment samples

It is difficult to quantify the proportions of other autotrophic algae than diatoms with precision. Counting cells was difficult because most of the living organisms were attached to sediment particles. However, based on subjective estimation, diatoms accounted for at least 90% of the biomass at station 1 on any sampling occasion. At station 2 other microalgae, e.g. cyanophytes and monads, occurred seasonally, mainly in late summer, but roughly 80--90% were diatoms. The cyanophytes were species from the genera *Merismopedia*, *Microcystis*, *Anabaena*, *Oscillatoria* and *Lyngbya*. During late summer patches of purple sulphur bacteria occurred near the shore at station 2.

Small pennate diatoms with a cell length in the range of 10--30 μ m dominated the diatom assemblages at both stations. They were mainly found attached to sand grains but motile species also occurred. The attached diatoms occurred in patches in cavities of the sand grains or protruded from the surface of the sand grains singly or in colonies (Plate 1). Colonies also occurred flat on the surface of the sediment particles. A dense cover of the protruding forms together with unidentifiable detritus-like matter surrounding the sand grains gave them a woolly appearance under the light microscope. Not all sand grains, however, possessed an epiflora.

The term 'epipsammic' (Round, 1965, 1981) will here be used in the broad sense of the word of all diatoms attached to sediment particles. The use of this term at the species level has been called in question by e.g. de Jonge (in press) and certainly a sharp distinction at species level is difficult e.g. because of varying adhesion capacity. Still I prefer to use the term epipsammic of species I most often found attached. The distinction is based on observations on (a) fresh sediment samples, (b) separate slides from sand and suspendible fractions (see Round, 1965) and (c) scanning electron micrographs of air-dried sand grains.

It was not possible to identify the small epipsammic forms in the cavities, but the cell shape suggested that they belonged to genera such as *Achnanthes*, *Cocconeis*, *Amphora* and *Navicula*. This was later confirmed by separate slides of the sand fraction and by SEM micrographs. The protruding cells could be identified as belonging to the genera *Fragilaria* and *Opephora* (Plate 1). Empty diatom frustules were also found attached to the sand grains, even after repeated washing to separate the sand fraction from the suspendible fraction.

The suspendible fraction (term adopted from Cadée and Hegeman, 1974) was built up of motile diatoms, empty diatom frustules, occasional detritus floccules, unidentified monads, micro- and meiofauna and also species usually found protruding from the sand.

The sand at station 1 gave a clean impression. The organisms were mostly found in the cavities of sand grains and there were few protruding cells and little suspendible matter. At station 2 protruding single cells and colonies were common, and there was more suspendible matter including detritus floccules which seem to be a favourable substrate for small diatoms and other unicellular algae. Ciliates were often found grazing on the floccules.

3.2. The microflora of the colonized glass slides

The aim of the colonization experiment was to study the species composition of the diatom assemblages, though many other organisms were also found on the slides. The degree of dominance of diatoms depended on season and incubation time.

Colonization during spring (Table 2, period 1) was slow. On only one of the eight immersed slides was colonization visible to the naked eye after 6 weeks, the cover being mainly made up of bacteria, cyanophytes (*Merismopedia* in particular) and juvenile stages of macroalgae. During summer colonization proceeded more quickly and the proportion of diatoms increased until *Cocconeis placentula* (mainly var. *euglypta*) was dominant in periods 4 and 5 in Table 2 (see also 3.4., Fig. 4). In early autumn (period 6) the dominance of diatoms was less pronounced: a dense bacterial cover made up the substrate on which the diatoms (including large-celled *Licmophora* spp.) grew together with juvenile forms of macroalgae. However, when the slides were left for an additional 4 weeks (period 7) *Cocconeis* species were again dominant and many cyanophytes were also present. The final colonization period (4 weeks) resulted in a sparse cover with diatoms as dominants.

The surface of the slides was not evenly colonized. The densest growth appeared as a brownish 2--20 mm broad zone at the sediment/water interface. A zone of macroalgae above this zone was observed on the spring slides (periods 1 and 2). Brown patches were also found at the edges of the slides (cf. Dickman and Gochner, 1978). These patches were partly due to precipitation (ferrous?) around cells of *Cocconeis*.

3.3. Dead cells in the diatom counts

The composition of the diatom assemblages will here be discussed on the basis of statistical analysis of relative abundance in oxidized diatom suspensions. Before presenting and interpreting the results it can be in place to discuss the drawbacks of this method.

Since diatoms attached to sand grains dominated the flora at the two sampling stations, I estimated the effect of the inclusion of dead cells by separating the sand from the suspendible fraction (Round, 1965), assuming that dead cells would mainly be found in the suspendible fraction (Table 3). Slides made from the suspendible fraction, however, had fewer cells than those made from the sand fraction although they were prepared from the same volume of sediment and in the same

Table 3. Relative abundance (%) of the commonest 10 diatom species in unfractionated samples and in sand and suspendible fractions separated by washing. Samples taken in April and September 1980. For abbreviations see Appendix 2.

Station 1

<u>Not fractionated</u>		<u>Sand fraction</u>		<u>Suspendible fraction</u>	
Ac hauc	28.1	Ac hauc	31.1	Ac hauc	19.5
Co plac	10.6	Co plac	11.8	Fr pinn	14.6
Ac lemm	8.6	Ac tene	8.9	Co plac	8.4
Ac tene	8.3	Ac lemm	8.9	Na sp 1	7.3
Na sp 5	5.6	Na sp 1	5.5	Op mart	7.0
Na sp 1	5.0	Na sp 5	5.3	Am exig	5.4
Am exig	5.0	Fr pinn	3.6	Ac lemm	5.1
Fr pinn	3.9	Am exig	3.6	Co sp 2	4.3
Na cryce	3.9	Na bisk	3.3	Na sp 5	3.8
Ni kuetz	2.6	Co sp 2	3.1	Ni kuetz	3.5
Ac hauc	21.1			Ac hauc	18.8
Ac lemm	11.0			Ac lemm	9.6
Ac tene	9.3			Fr pinn	9.6
Co plac	7.6			Na sp 1	6.6
Na sp 1	7.6			Co plac	4.3
Na hans	4.2			Na hans	4.3
Na cryce	3.7			Op mart	4.0
Na sp 5	3.6			Na grac	4.0
Am exig	3.4			Am exig	3.6
Ni kuetz	2.5			Ni kuetz	3.6
Ni frust	2.5				

Station 2

<u>Not fractionated</u>		<u>Sand fraction</u>		<u>Suspendible fraction</u>	
Fr pinn	32.6	Fr pinn	20.4	Fr pinn	44.8
Ni kuetz	14.1	Ac lemm	15.2	Ni kuetz	15.3
Op mart	9.6	Ni kuetz	14.5	Op mart	8.8
Ac hauc	8.5	Ac hauc	10.3	Ac lemm	4.5
Ac lemm	7.0	Ni frust	8.4	Na sp 5	3.7
Ni frust	3.8	Na bisk	6.7	Ac hauc	2.9
Na sp 5	2.5	Na sp 5	4.2	Op sp 1	2.5
Op sp 1	2.3	Op mart	2.7	Ni frust	2.3
Na cryce	1.9	Na cryly	2.7	Na bisk	2.2
Na phyll	1.5	Na sp 1	2.5	Am sp 1	2.2
Fr pinn	20.7			Fr pinn	40.1
Ac lemm	12.1			Op mart	8.6
Op mart	11.8			Sy tabu	7.3
Na bisk	8.9			Na phyll	6.4
Ac hauc	8.4			Ni kuetz	5.1
Ni kuetz	6.4			Na bisk	4.8
Na sp 5	5.1			Ac lemm	4.1
Ac tene	3.8			Ac hauc	3.2
Ni frust	3.0			Na men1	2.5
Na cryce	2.8			Co plac	1.6

way. This indicated that there was only a small proportion of dead cells. At station 2, however, more fragments of frustules were found than at station 1.

Table 3 shows that the proportions of species in the unfractionated sample and the sand fraction are about the same for station 1. At station 2, the difference is greater mainly because the protruding life forms (*Fragilaria* spp. and *Opephora* sp.) easily become detached when the sample is shaken. These results suggest that the inclusion of dead cells is not a serious source of error in the Falsterbo material.

3.4. Numerical analysis of the diatom assemblages

The ordinations and classifications were run on the whole data set as well as on subsets corresponding to station or substrate. Both relative abundance and presence/absence data were used. The relative abundance data proved to be more informative indicating that the structural variations were due to differences in the proportions of species rather than presence/absence (Table 4).

In the classifications the fusion of units in the subsets was stopped at the similarity ratio c. 0.80. This level was chosen subjectively. The results of all classifications are shown in a single synoptic table (Table 5).

3.4.1. Preliminary classification and ordination

In a preliminary classification of the whole data set the fusion of samples was stopped when three clusters remained. These clusters corresponded fully, with the exception of one sample, to the three hypothetical diatom assemblages, viz. the sediment flora of station 1 (I), the sediment flora of station 2 (II) and the diatom flora of the glass slides at station 2 (III). The similarity ratio (range 0--1) between the clusters was 0.419 between I and II, 0.203 between I and III and 0.168 between II and III. These three groups are re-

Table 4. The percentages of extracted variance for the first four dimensions based on relative abundance and presence/absence in ordinations of the data sets.
 n = number of samples, c.a. = coefficient of alienation, a measure of the alienation by reduction of dimensions from the original distances.

Data set	n	Relative abundance data		Presence/absence data	
		Extr. var.,%	c.a.	Extr. var.,%	c.a.
Total	67	90.8	0.08	30.9	0.27
Sand, St.1	26	72.5	0.15	35.9	0.25
Sand, St.2	26	85.2	0.08	30.6	0.29
Glass	23	95.6	0.04	53.5	0.22

ferred to as cluster groups and are separated by solid lines in the synoptic TABORD table (Table 5). The term cluster will be used for the groups created in the classification of the subsets corresponding to the three cluster groups.

The first four dimensions in the preliminary ordination extracted 55.7%, 27.6%, 5.1% and 2.4% respectively of the total variance (Fig. 3). The variation along the first axis (dimension) is due to the variation within cluster group III (colonized glass slides). The main difference between the two sediment assemblages occurs along axis 2.

The three cluster groups produced by the preliminary classification differed because of the different proportions of shared species. However, these groups are sufficiently distinct to be termed associations and are named after the dominating (mean RA $\geq 10\%$) constant species occurring at least once with a RA $\geq 30\%$:

- I an *Achnanthes hauckiana*-association = sediment flora at station 1
- II a *Fragilaria pinnata*-*Opephora martyi*-association = the sediment flora at station 2
- III a *Cocconeis placentula*-association = the diatom flora on colonized glass slides at station 2

Table 5. Phytosociological table produced by the TABORD program. Cluster groups refer to the clusters produced by the preliminary classification of the whole data set. Clusters are indicated by numbers on the top line according to separate classifications of the subsets. The digits = relative abundance classes. (-) = RA $\leq$ 2% and (B) RA = $\geq$ 40%. For abbreviations see Appendix 2. Only part of the table is shown.

Cluster group	I	II	III
	1111111111111111	222222223344444444445555555666666	7888899999991010
Ac leva	-	-	-
Am oval pedi	-----1-----11	-	-
Ct adha	-----1-----	-	-
Co sp 2	-----1-----	-	-
Na hans	----1 - 1-----22 1-----	-	31 -
Na incer	-----1-----	1--2-3-----	2 -- - - -
Op schu	-----	-	-
Ac delic	-----1-----	-	-
Ac hauc	A99AAA9A9AA9AA8	777888888574444343424336663426887688	32-132--1--
Ac lemm	311423322212223	544553334433365244332422552-1422346	-----2-1--1--
Ac tene	3-32311112322311	4223233321-----111--1-11	1- - - - -
Am exig	2241-23142211--	11111122-----1-1-----3-----111	11 11 61--5--1
Am hols	-----	-	-
Co plac	3356324557663732	322335463-----1-----	38886BBBBABBBBB
Co scut	-----11-----	-----2-----1-----	4 -1- 211- --
Fr pinn	--5-1-311--3--	1432321466489989899A9A5576797368376	756976748751 54
Na amphi	--- -1- -1-----	571 1-----1-121-4-2-1-1-1	--- - - - -
Na arni	-----	-	-
Na bisk	- - --1-1-----	2132-1-31-11432332111-----2-----3412	-1-1-- - 1
Na cryce	1--1-32--11-2--11	-----1111-----1-----11-----11--1-2-----1--	--- - - - -
Na cryly	33-1311122121121	-----1-----2- 1- --1- -	-
Na grac	-----	-	-
Na phyll	-----	-----11-----1--21-1-122- -- - -	-
Na sp 5	1 1-----1-----	121--1211211-12 111112121231--421-12--	- - -
Na sp 1	22116-1523154333	3223212121--11--1-----2-----1112--	- - -
Ni frus subs	1-----21-----	1--i- 11--14-1111131--21-132-136643	-1-1--1-- 1-
Ni kuetz	-----	1--11-11343442352665574 123- -2 111168352221 1-----	-
Op mart	-121-111-1123111	-22122--16563753543343377898AA662342	121124-131--1-
Sy tabu	--- 1-----	-----1-----1-----	-1-- 1112 --
Co dimi	-----	-	-
Am tene	-----	-	-2-- -111- -
Fr vir subs	-- - - -	-----1-----11- 11-1 1--	- - - - -
Na bahu	-----	-	-
Ni kuetz exil	- - - - -	1-311 12--1 1-11- 1113-11	-
Am coff	-- - - -	-	1-----
Na sali	1 --- - -	-----11-----	-2
Am ablu	-----	-----	-1 -- - - 2
Ni micro	- - -	-	-
Am coff perp	- - -	- 1 - - 1 - -	2--1-- - - -
Op sp 1	-- - - -	- -1 --11 - -	1--1----- - -
Am sp 3	-----	-	--11 --1
Fr sp 1	6	-	- - - -
Co stau	-- -1 4--	-	- - - -
Ac brev	-----	-	---3

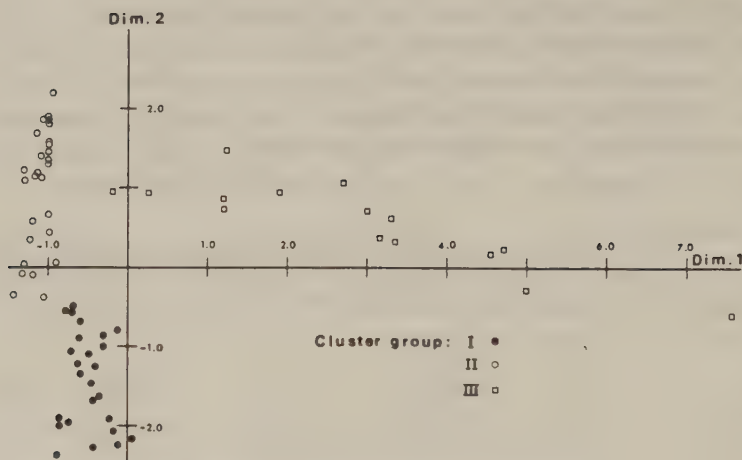


Fig. 3. Diagram of the preliminary ordination showing dimension 1 and dimension 2. The cluster groups correspond to sediment from station 1 (I), sediment from station 2 (II) and colonized glass at station 2 (III).

3.4.2. Structure of the diatom flora of the sediment (Cluster groups I and II)

Station 1 (=cluster group I)

A more detailed classification of the samples in cluster group I (the *Achnanthes hauckiana*-association) revealed two distinct, though similar (SR 0.827), clusters (1 and 2 in Table 5) corresponding to the two sampling periods: 16 samples from Period I (one sample fell into cluster group II) and 9 samples from Period II. The main difference between the clusters was due to a change in the proportions of the constant species such as *Navicula cryptolyra*, *Navicula* cf. *biskanteri* and *Nitzschia kuetzingiana* (Tables 5 and 6).

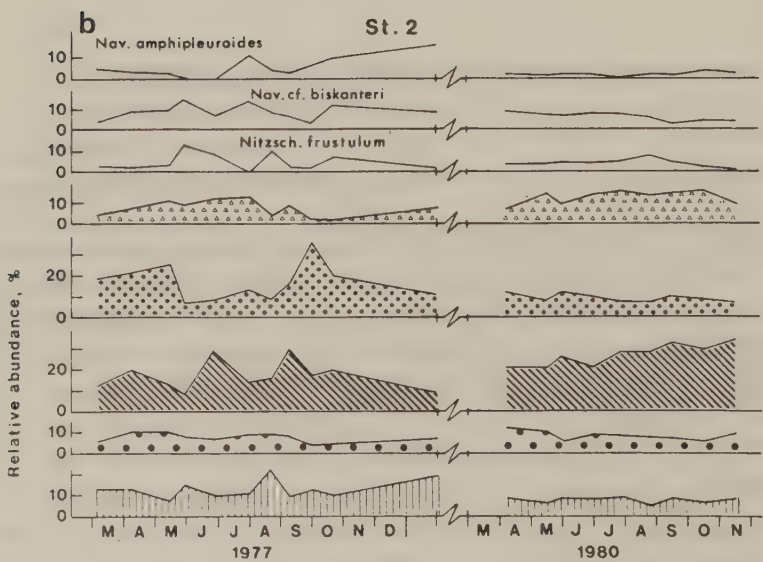
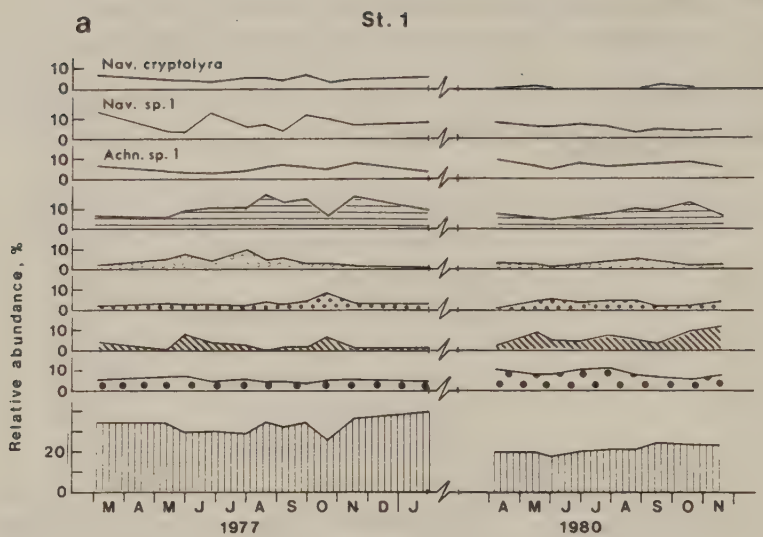
No clusters indicating clear seasonal changes in the diatom community were produced. However, although some variation was found in the relative abundance of the commonest species (Fig. 4a) no marked successional trend as found for phytoplankton communities was observed. In a few cases normally

Table 6. Mean relative abundance (RA, %) of the commonest species (cumulatively up to about 50% of the total cell count) in clusters 1 and 2 in cluster group I = sediment at station 1.

<u>Cluster</u>	<u>Cluster size</u> (samples)	<u>Species</u>	<u>RA, %</u>
1	16	<i>Achnanthes hauckiana</i>	31.5
		<i>Cocconeis placentula</i>	10.1
		<i>Navicula</i> sp. 1	6.4
		<i>Achnanthes lemmermannii</i>	5.4
2	9	<i>Achnanthes hauckiana</i>	21.1
		<i>A. lemmermannii</i>	8.8
		<i>Cocconeis placentula</i>	7.8
		<i>A. cf. tenera</i>	6.7
		<i>Navicula</i> sp. 1	5.1

rare species had a noticeably higher relative abundance: *Fragilaria* sp. in April 1976 and *Cocconeis scutellum* var. *stauroneiformis* in April 1978 (Table 5). This may, however, be the result of contamination by allochthonous species, e.g. epiphytes.

Dimension 1 in the ordination extracted almost half of the total variance reflecting the change in species composition from Period I to Period II (Table 7, Fig. 5). The variation during Periods I and II is diagonal to the axes 1 and 2. Fig. 5 also shows a similar trend in material collected in late summer and autumn in both 1977 and 1980 i.e. a tendency to lie below the dimension 1 axis and to tend to the right.



C Colonization, St.2

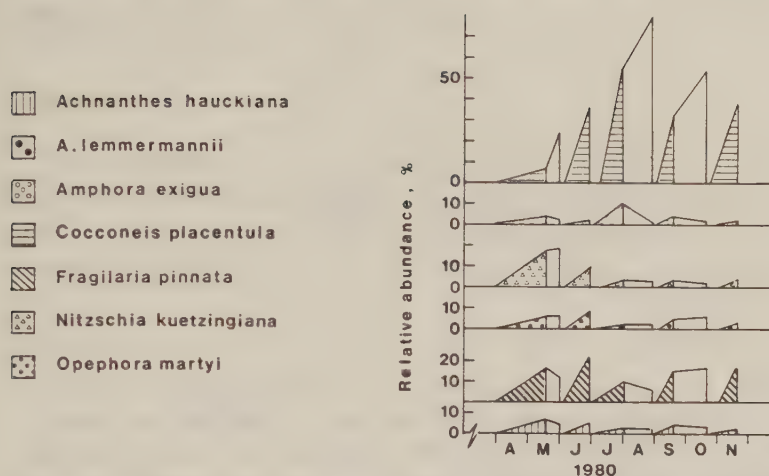


Fig. 4. Relative abundance of selected commonest species during 1977 (Period I) and 1980 (Period II).

(a) in sediment at station 1; (b) in sediment at station 2; (c) on colonized glass slides at station 2 (only 1980).

Triangles with shading and dots represent 4-week colonization and unfilled part additional 4-week colonization.

Table 7. Percentage variance extracted by the first 4 dimensions in the quantitative ordinations of the three cluster groups.

Cluster group	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Total
I	45.8	10.3	8.8	7.6	72.5
II	39.5	29.7	9.8	6.2	85.2
III	84.7	5.1	3.3	2.5	95.6

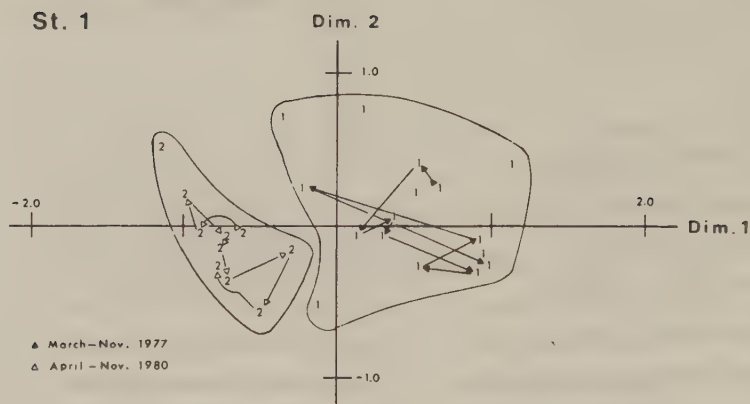


Fig. 5. Ordination (dimensions 1 and 2) of the samples from sediment at station 1. Clusters produced by the classification program TABORD are indicated by numbers. Arrows show the direction of change for the years 1977 and 1980.

Station 2 (=cluster group II)

Four final clusters were produced by the classification when fusion was stopped at SR 0.806 (clusters 3 to 6 in Table 5). The main differences between the clusters were due to change in relative abundance for the commonest constant species (Tables 5 and 8). Cluster 4, the largest and most homogeneous group (internal average SR 0.929), included all samples from 1980 (Period II) and three samples from Period I. Samples from 1977 (10 out of 17 Period I samples) were scattered in all four clusters indicating wider variation in the material collected during Period I.

Dimensions 1 and 2 in the ordination accounted for 39.5% and 29.7% of the total variance respectively (Table 7). In Fig. 6 the samples and clusters tend to be grouped in a horseshoe-shaped formation, which is typical of the kind of ordination analysis used here. The wider variations of the Period I samples, as seen by changes from one cluster to

another in the ordination diagram (see also Fig. 4b), may partly reflect spatial variation since the position of the sampling station was not permanently marked during Period I.

Table 8. Mean relative abundance (RA, %) of the commonest species (cumulatively up to about 50% of the total cell count) in clusters 3 to 6 in cluster group II = sediment at station 2.

<u>Cluster</u>	<u>Cluster size</u> (samples)	<u>Species</u>	<u>RA, %</u>
3	2	<i>Achnanthes hauckiana</i>	15.8
		<i>Navicula amphipleuroides</i>	12.7
		<i>Opephora martyi</i>	11.7
		<i>Fragilaria pinnata</i>	11.1
4	12	<i>Fragilaria pinnata</i>	26.7
		<i>Nitzschia kuetzingiana</i>	12.0
		<i>Opephora martyi</i>	9.6
5	7	<i>Opephora martyi</i>	24.7
		<i>Fragilaria pinnata</i>	17.2
		<i>Achnanthes hauckiana</i>	10.5
6	5 (6) *	<i>Achnanthes hauckiana</i>	19.7
		<i>Fragilaria pinnata</i>	14.4
		<i>Opephora martyi</i>	7.7
		<i>Achnanthes lemmermannii</i>	7.7

* Cluster size 6 including one sample from station 1.

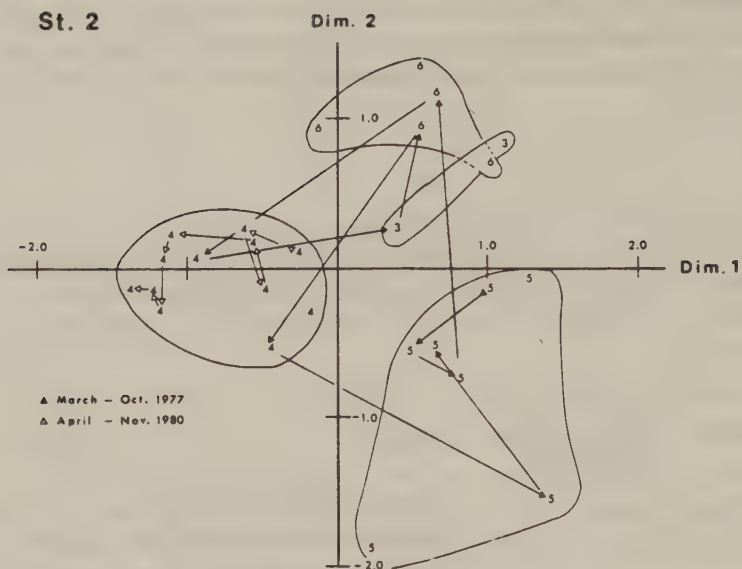


Fig. 6. Ordination (dimensions 1 and 2) of the samples from sediment at station 2. Clusters produced by the classification program TABORD are indicated by numbers. Arrows show the direction of change for the years 1977 and 1980 respectively.

3.4.3. Structure of the diatom flora of the glass slides (Cluster group III)

The numerical analyses of the colonizing diatom flora were based on a matrix constructed from data obtained from two replicate slides together with the mean of the replicates on each sampling occasion. Four clusters remained when fusion was stopped at SR 0.810 (clusters 7 to 10 in Table 5). The disparity of the replicate slides was demonstrated by the fact that in half of the cases the duplicates were not found in the same cluster. In Table 9 only the mean of the replicates are included.

The species composition of the first colonization period (cluster 7) was dominated by the same two species as the dia-

Table 9. Mean relative abundance (RA, %) of the commonest species (cumulatively up to about 50% of the total cell count) in clusters 7 to 10 in cluster group III = colonized glass slides at station 2, 1980.

Cluster	Incubation period	Species	RA, %
7	1 (04-06--05-18)	<i>Fragilaria pinnata</i>	16.0
		<i>Nitzschia kuetzingiana</i>	12.8
		<i>Cocconeis scutellum</i>	8.8
		<i>Cocconeis placentula</i>	6.6
		<i>Achnanthes hauckiana</i>	6.2
8	2 (04-06--06-01)	<i>Cocconeis placentula</i>	22.6
		<i>Nitzschia kuetzingiana</i>	15.0
		<i>Fragilaria pinnata</i>	11.9
9	3 (06-01--06-29)	<i>Cocconeis placentula</i>	39.1
	6 (08-24--09-14)	<i>Fragilaria pinnata</i>	16.4
	7 (08-24--10-19)		
	8 (10-19--11-16)		
10	4 (06-29--07-27)	<i>Cocconeis placentula</i>	66.3
	5 (06-29--08-24)		

tom flora of the sediment at the same site in 1980 (cluster 4 in cluster group II), but differed in the high proportion of *Cocconeis* individuals (see Tables 8 and 9). The other three clusters were dominated by *Cocconeis placentula* (mainly var. *euglypta*). The highest relative abundance for *C. placentula* (95.0%) was found for a slide incubated from July to August (8 weeks) (Fig. 4c).

The first dimension in the ordination accounted for 84.7%, i.e. as much as the four dimensions together for the sediment samples at the same station (Table 7). This indicates that the variance of the diatom assemblage of the glass substrates was less multidimensional than in the natural substrate (=sand). The first dimension reflects the succession gradient

towards the dominance of *Cocconeis placentula* et var. in summer (Fig. 7). In autumn there was a trend in the opposite direction along the same axis reflecting the decreased importance of *Cocconeis* (Fig. 7, see also Fig. 4c). The scatter along the other axes is due to the differences between the replicate slides.

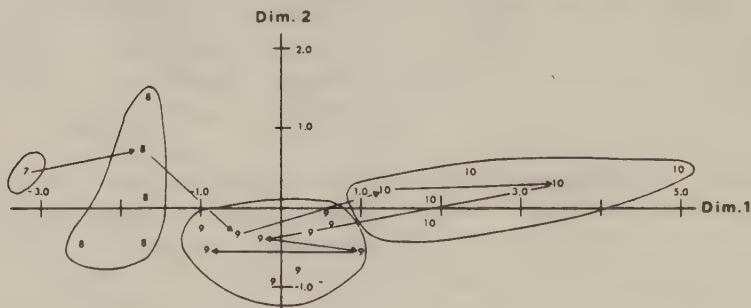


Fig. 7. Ordination of colonized slides from station 2, 1980. The numbers 7--10 denote the clusters produced by the classification program. Arrows show the direction of change.

3.4.4. Diversity of the diatom assemblages

Diversity (H') and evenness (J') in the diatom associations in the sediment were high and stable (Figs 8A, B). The values of H' fluctuated between 3.28 and 4.55. A slight tendency towards lower values during autumn was observed in 1980. Evenness ranged from 0.64 to 0.81 (Figs 8A, B).

On the colonized glass slides diversity and evenness fluctuated markedly (Fig. 8C). Both indices (H' and J') decreased in summer as a result of the invasion of *Cocconeis placentula*. Note also the higher index values for 4-week slides (September) compared with the 8-week slides (October).

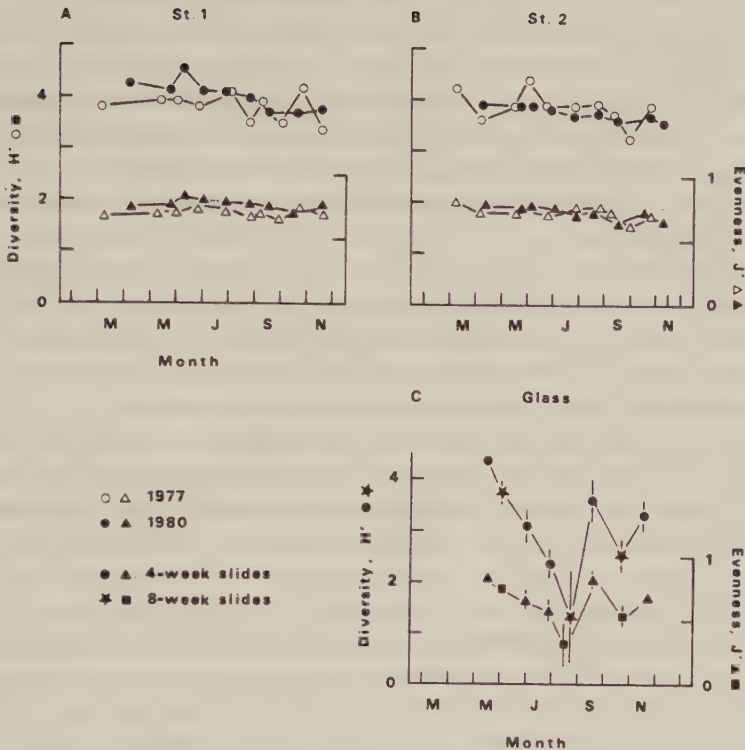


Fig. 8. Diversity (H') and evenness (J') for (A) sediment samples from station 1; (B) sediment samples from station 2; (C) samples from colonized glass slides at station 2 (only for 1980). Range for replicates shown for colonized slides.

3.5. Life forms and cell size

Before summarizing the main features of the three associations two more aspects will be added to the characterization, viz. the life form and size of the diatom species. On the basis of observations of the fresh collections, the separate permanent slides of the sand and of the suspendible fraction and SEM micrographs of air-dried sand (cf. Plate 1) five life forms could be distinguished:

- (a) Flat, attached diatoms in the cavities of sand grains, cell length 5--20(-30) μm (e.g. species of *Achnanthes*, *Cocconeis*, *Navicula* and *Amphora*; see Plate 1).
- (b) Attached single individuals or colonies of usually rapheless species protruding from the surface of sand grains (species of *Fragilaria* and *Opephora*; see Plate 1).
- (c) Small (<30 μm) motile species of the genera *Navicula* and *Nitzschia*.
- (d) Large (>30 μm) motile species of e.g. *Navicula*, *Nitzschia*, *Mastogloia*, *Pleurosigma*, *Amphora*, *Caloneis*, etc.
- (e) Large, usually apically attached araphideans and monoraphideans, e.g. *Licmophora* spp., *Synedra* spp. and *Achnanthes brevipes* (attached by a mucus foot).

The life forms are not distinct (van den Hoek et al., 1979, de Jonge, in press). It is difficult to judge whether a small *Navicula* species, for example, lives most of its time on a sand grain or in the interstitial space. Life forms (b) and (c) were moreover often found entrapped in detritus floccules, thus occasionally belonging to the suspendible fraction. However, although this division into life forms may seem artificial, it is useful in describing the structure of the different associations.

Since cell counts may favour small species, the RA values were multiplied by a rough mean value of the cell volume of a species and the percentages were recalculated. To avoid time-consuming recalculations of the whole of the data only constant species of each association (=cluster group) were treated, i.e. 88%, 90% and 81% of the mean total cell count for each of the three associations. These species were grouped into 4 size classes, corresponding to the subjective groups 'very small' (size class 1), 'small' (size class 2), 'medium-sized' (size class 3) and large (size class 4):

Size class 1: < 50 μm^3
 2: 50 - 100 μm^3
 3: 100 - 250 μm^3
 4: > 250 μm^3

Table 10 compares the relative abundance of the commonest species of each association based on cell number and cell volume. *Achnanthes hauckiana* dominates the sand at station 1, even when cell volume is taken into account. However, large rare species replace small common species when volume is considered. The true importance of these large species is questionable since *Synedra tabulata*, for instance, was rarely a living constituent of the sediment flora but can be classified as detached epiphyton. Cell counts gave an overestimate of the significance of the small *Fragilaria pinnata* (Table 10) whereas species such as *Navicula phyllepta* and *Fragilaria virescens* var. *subsalina* have previously been underestimated. If cell volume is taken into account the *Fragilaria pinnata*-*Opephora martyi*-association should preferably be called the *Opephora martyi*-association. On colonized glass slides *Synedra tabulata* on the other hand is of actual significance since living cells were frequently observed growing on the glass slides.

Fig. 9 shows that life form (a) dominates both the sediment at station 1 and the colonized glass slides. Fig. 10, however, shows that the assemblages on glass consist of larger cells, partly because species common to both substrates were larger on glass, and partly because of the different life forms: large epiphytic species such as *Lichmophora* spp. and *Achnanthes brevipes* (life form (e)) were more abundant on glass (Fig. 9). In the *Fragilaria pinnata*-*Opephora martyi*-association in the sediment at station 2 life forms (a) and (b) are about equally represented (Fig. 9).

3.6. Summary of the characteristics

The characteristics of the three associations are summarized in Table 11. In spite of the quantitative differences, the two sediment associations share the main features of the community. Both associations are dominated by small species attached to sand grains (epipsammic), accounting for about 70--80% of the total cell volume of diatoms (Table 11; Figs 9 and 10). 86% of the constant species at station 1 and 77%

Table 10. Mean proportions of the 10 commonest species in the three associations (cluster groups), expressed as relative abundance based on cell number and cell volume. For size groups and life forms see text. For abbreviations of species see Appendix 2.

Station 1

	Ra, % of cell number	Size group	Life form		RA, % of cell volume	Size group	Life form
Ac hauc	31.1	2	a	Ac hauc	25.9	2	a
Co plac	10.2	2	a	Am exig	10.2	3	a,d
Ac lemm	7.6	2	a	Co plac	7.2	2	a
Na sp 1	6.4	3	a	Na sp 1	6.9	3	a
Ac tene	6.1	1	a	Ac lemm	5.5	2	a
Fr pinn	4.6	1	b	Op mart	5.2	3	b
Am exig	4.3	3	a,d	Sy tabu	4.8	4	e
Op mart	4.0	3	b	Am hols	4.2	4	d
Na cryl	3.3	2	a	Na phyl	2.9	4	d
Na sp 5	2.7	2	a	Co scut	2.8	4	a

Station 2

Fr pinn	21.5	1	b	Op mart	20.0	3	b
Op mart	14.8	3	b	Ac hauc	11.0	2	a
Ac hauc	12.8	2	a	Na phyll	7.0	4	d
Ac lemm	8.4	2	a	Fr vir subs	6.6	4	b
Ni kuetz	6.9	2	c	Ac lemm	6.3	2	a
Ni frust	5.1	1	c	Ni kuetz	5.9	2	c
Na bisk	4.6	2	a	Fr pinn	4.5	1	b
Na sp 5	3.7	2	a	Am coff	4.2	4	d
Na amph	3.3	2	a	Am exig	4.1	3	a,d
Na sp 1	2.4	2	c	Sy tabu	4.1	4	e

Glass slides

Co plac	48.9	3	a	Co plac	59.3	3	a
Fr pinn	16.7	1	b	Sy tabu	15.4	4	e
Ni kuetz	7.5	2	c	Am exig	8.4	4	a,d
Am exig	4.2	4	a,d	Am coff	4.7	4	d
Op mart	4.2	3	b	Ni kuetz	3.1	2	c
Ac hauc	3.9	2	a	Op mart	2.5	3	b
Sy tabu	1.9	4	e	Fr pinn	2.2	1	b
Na cryce	1.8	3	a,c	Ac hauc	2.1	2	a
Am coff	1.7	4	d	Na cryce	1.2	3	a,c
Ac lemm	1.7	2	a	Am tene	1.1	3	a

at station 2 had a mean cell length of $<20\text{ }\mu\text{m}$. The main differences between the two sediment associations were due to different proportions of shared species. According to Simonsen

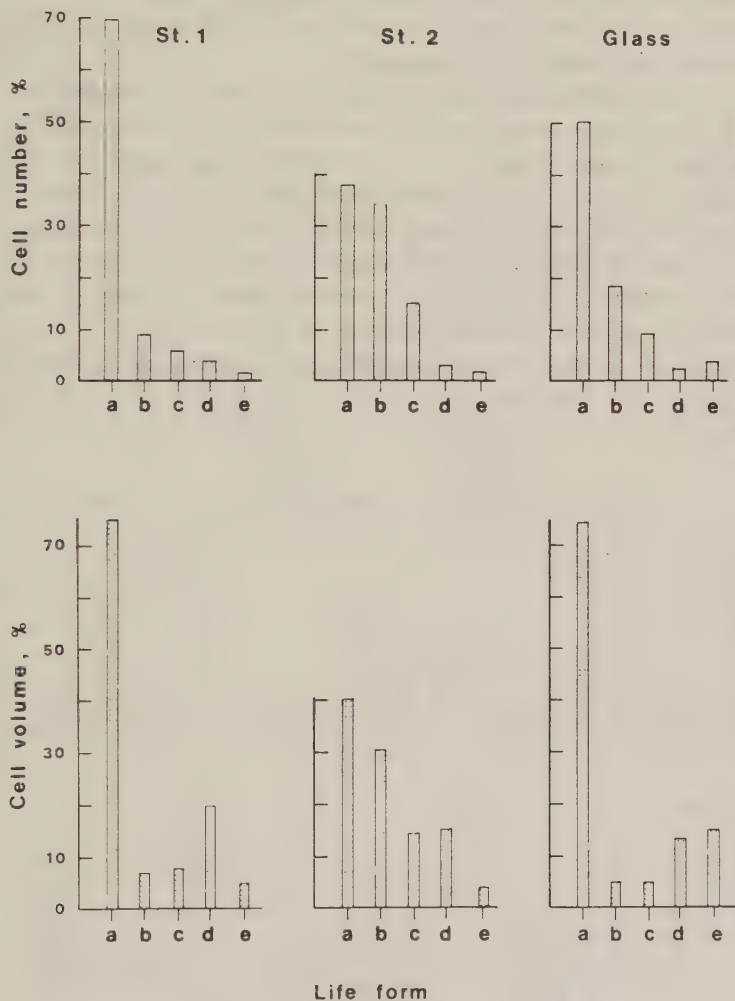


Fig. 9. Proportions of different life forms in sediment and on colonized glass based on cell number and cell volume. Sum of the columns may exceed 100%, because some species are included as two life forms. For identification of life forms see p. 96. Only constant species are included.

(1962), the commonest species can be characterized as strongly euryhaline (mostly euryhaline mesohalobes and pleioeuryhaline oligohalobes).

My investigation (2 years) did not reveal any great seasonal variation in the composition of the diatom flora. Community diversity and evenness also changed very little and were high throughout the investigation periods.

There was a pronounced successional change in the diatom flora on the glass substrate by contrast with the stability of the sediment flora. However, the species composition found on the slides in early spring when colonization rate was slow resembled that of the sediment (see e.g. Fig. 3). The differences in species composition were mainly due to the dominance of *Cocconeis placentula* et var., a species rarely found in the sediment at station 2 though common at station 1.

The diversity of the diatom assemblages colonizing the glass slides was less than and more variable than for the natural substrate.

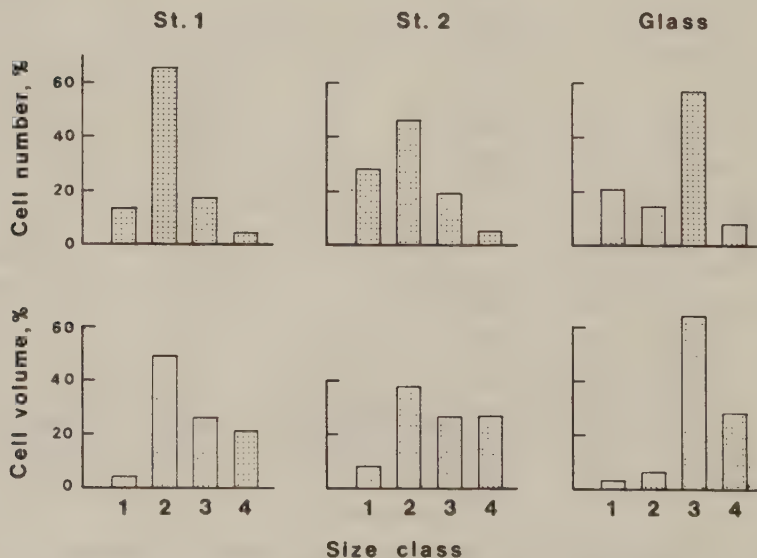


Fig. 10. Proportions of size classes based on relative abundance calculated from cell numbers and from cell volume. Only constant species are included. For size classes see p.96.

Table 11. Characteristics of the three diatom associations. For comparison of results from the natural and artificial substrates Period II at station 2 is treated separately since colonization was confined to this period.

Association	Periods I and II		Period II	
	Achnanthes hauckiana	Fragilaria pinnata-Opephora martyi	Fragilaria pinnata-Opephora martyi	Cocconeis placentula
Station	1	2	2	2
Substrate	sandy sediment	sandy sediment	sandy sediment	glass
Sampling occasions	26	26	9	8
Number of taxa ¹⁾	108	112	77	80
Number of constant spp.	29	26	28	15
Dominant taxa (RA, % >10%)	5	7	4	4
Diversity, H'	3.52 - 4.55 ($\bar{x}$ =3.95, CV=6.8%) ²⁾	3.28 - 4.40 ($\bar{x}$ =3.85, CV=5.9%)	3.61 - 3.95 ($\bar{x}$ =3.80, CV=4.3%)	0.40 - 4.40 ($\bar{x}$ =2.99, CV=32.6%)
Evenness, J'	0.64 - 0.81 ($\bar{x}$ =0.73, CV=5.9%) ²⁾	0.64 - 0.80 ($\bar{x}$ =0.73, CV=5.4%)	0.67 - 0.76 ($\bar{x}$ =0.72, CV=4.3%)	0.12 - 0.85 ($\bar{x}$ =0.64, CV=28.4%)
Dominant life form	a	a, b	a, b	a
Dominant size group	2	2	2	3

1) Taxa included in the numerical analysis.

2) Arithmetic means of logarithmic functions calculated, although not recommendable, to demonstrate the differences in variation by CV (coefficient of variation).

4. DISCUSSION

4.1. Spatial distribution

When discussing the structure of the microphytobenthic diatom community both geographic distribution and habitat must be taken into account. The two stations investigated represent a restricted area in a transition zone between the Baltic and the Kattegat. The decreasing salinity southwards gradually limits the distribution of marine organisms in Öresund, e.g. marine macroalgae (von Wachenfeldt, 1975). The same benthic diatom species have been found in sediment samples from Nivå Bay, Denmark, (M. Gargas, 1980) and from 14 localities along the Swedish coast of Öresund (Sundbäck, 1979). This suggests that the bulk of the diatom flora on semi-exposed (wave action) sandy bottoms of shallow water along the coasts of Öresund consists of the same species, which according to Simonsen (1962) and Wolf (1982) are characterized as strongly euryhaline.

Möller (1950) investigated the benthic diatom flora of the Prästö Fjord, western Baltic, and mentions *Achnanthes hauckiana*, *Fragilaria pinnata*, *Nitzschia frustulum* var. *subsalina* and *Opephora olsenii* among 32 species he considered as characteristic of the area. He characterized the species as euryhaline, which means that other ecological factors have a more dominating effect than salt content. The *Navicula cryptocephala*-*Amphora coffeaeformis*-association mentioned by Pankow (1980) as a widespread association of shallow waters in the southern Baltic was not, however, recognized in this investigation. The species included in the association were also found in the Falsterbo area, but they were not very abundant probably because of different degree of exposure to wave action and differences in sediment type (see discussion below).

A dense epipellic flora consisting of large motile diatom species, characteristic of tidal flats (e.g. van den Hoek et al., 1979) was not observed either. However, an epipellic diatom flora was found in a shallow sheltered muddy bay in southern Öresund (Foteviken) and also developed in aquaria with natural sediment from Lomma Bay (middle part of Öresund) kept in the laboratory (Sundbäck, unpublished).

Between 70 and 80% of the diatom biomass at the two Falsterbo stations was estimated to be made up of life forms attached to sand grains (epipsammic). These values agree fairly well with the proportion of assimilated ^{14}C in the sand fraction, i.e. 80--85% (Sundbäck, 1983) and are within the range reported by Hunding (1971) for the sandy shore of a lake and by Gargas (1970) for Nivå Bay, the Danish coast of Öresund. Small cell size has been found to be characteristic of epipsammic diatoms both in fresh-water (Round, 1965) and marine habitats (Amspoker, 1977), although larger species have also been found attached to sand grains (Round, 1979). Large cells apparently cannot attach themselves to the surface of the sand grains as effectively as small cells, nor will they fit so easily in the cavities.

Most of the investigations on the structure of sediment associated diatom communities have been conducted on tidal flats (e.g. Brockmann, 1950; Colijn and Nienhuis, 1978), estuaries (Amspoker and McIntire, 1978; Admiraal and Peletier, 1980; de Jonge, in press) or salt marshes (Sullivan, 1975, 1982; Cook and Whipple, 1982). My study area differs from these localities in two important aspects: (a) lack of regular tidal emergence and mixing of the sediment by tidal currents and (b) the lack of strong seasonal changes in salinity, typical of estuaries. Nevertheless many of the commonest epipsammic species (e.g. *Achnanthes hauckiana*, *Fragilaria pinnata*, *Navicula biskanteri*) were the same as those described for sandy tidal areas in different parts of the world (cf. Rao and Lewin, 1976; Colijn and Koeman, 1975; Colijn and Dijkema, 1981). Many species, both attached and motile are also found in salt marshes (Sullivan, 1981). The prevalent low salinity of the study area, however, restricts the presence of the genera of more saline waters, for example *Cymatosira*, *Raphoneis* and *Striatella*, reported as frequently occurring in sandy habitats on tidal flats (e.g. van der Werff, 1960; van den Hoek et al., 1979). Species belonging to these genera are found in the northern Öresund (Sundbäck, 1979) but in the Falsterbo area, there are more species characteristic of water

of low salinity than of water of higher salinity, i.e. the commonest species comprise more species characterized as euryhaline oligohalobes than euryhaline polyhalobes (see Simonsen, 1962).

Colijn and Nienhuis (1978) found that benthic diatoms on a shallow in the German Wadden Sea had a distinct pattern of distribution, which agrees with the distributional pattern of the habitat types. The sandy habitat of the geographically close localities investigated here, apparently differs enough to create at least quantitatively different associations. The sediment at station 2 is more favourable for benthic microflora (Sundbäck, 1983) and especially for diatoms projecting from the surface of the sand particles. How do the habitats differ and what is known about the factors controlling the spatial distribution of benthic diatoms?

So far, little experimental work has been done on the factors controlling the distribution of benthic diatoms, in particular epipsammic diatoms. Admiraal (1977a, b), Admiraal and Peletier (1979, 1980) and Admiraal et al., (1982) mainly used epipelagic species or mixed populations in their ecophysiological experiments, which is natural since epipsammic species are more difficult to isolate.

Some information on attached diatoms is provided by in situ investigations. It was suggested that factors such as salinity, exposure to emergence, light intensity and temperature together with biological interactions regulate the distribution of littoral diatoms (on PVC plates) in the Yaquina Estuary, Oregon (McIntire and Overton, 1971; Moore and McIntire, 1977; McIntire, 1978). Admiraal and Peletier (1980) found that distribution in the Ems estuary was related to salinity and possibly also to organic load. Salinity cannot account for the difference between the two associations in the Falsterbo area, the difference in salinity being negligible (Sundbäck, 1983). Moreover, the bulk of the diatom flora consisted of species known as being strongly euryhaline. Temperature, nutrients and light conditions did not differ sufficiently to create different habitats (Sundbäck, 1983). Nor can local discharges explain the difference, although there was a locally restricted accumulation of

decaying organic matter (macroalgae) during late summer at station 2. Little is, however, known about biological interactions between the diatom populations and other organisms. There was some difference in amount of macrofauna (Sundbäck, 1983), but the presumed greater grazing effect at station 2 would hardly favour protruding cells, which would be easiest to remove by browsers, such as the amphipod *Bathyporeia pilosa*. Only a few of these amphipods, shown to be able to affect the diatom populations (Sundbäck and Persson, 1981), were found on the sampling occasions.

However, the sampling stations differed in degree of exposure to water movement resulting in different sediment structure. The difference in grain size implies a difference in the amount of interstitial space available to microflora and also affects the penetration of light into the sediment (Haardt and Nielsen, 1980). The microtopography of the sediment particles may also influence the type of epipsammic flora that can inhabit them, an aspect that needs to be further investigated. De Jonge (in press) recently found that most of the pennate diatom cells were found in or on silt coatings on sand grains, i.e. not directly attached to sand grains.

It is suggested that degree of exposure to water movement accounts for the difference between the associations. This difference concerns life form rather than what individual species are involved. Protruding individuals and colonies (life form (b), cf. Round, 1971) together with small motile individuals (life form (c)) account for a greater part of the diatom flora at station 2. The lower chlorophyll *a* values found at station 1 (Sundbäck, 1983) can be explained by the more exposed position of the station, i.e. the mixing of the sediment by water movement restricts the presence of life forms that are less firmly attached.

That exposure to wave action is the main factor controlling the species composition of benthic diatoms has also been indicated by other investigations (Colijn and Nienhuis, 1978; Colijn and Dijkema, 1981). De Jonge (in press) has demonstrated the influence of exposure on diatom populations on sand grains

in the Ems-Dollard Estuary. Amspoker and McIntire (1978) calculated by multivariate analysis that sediment-associated diatoms were distributed not only according to salinity but also according to sediment structure in the Yaquina estuary. They did not find, as in earlier investigations with colonized artificial substrates (e.g. McIntire and Overton, 1971) any marked influence by light, temperature or exposure to emergence. They suggested that the sediment flora is less affected by the physical and chemical changes compared for example, with the epiphytic flora, because of the properties of the sediment (e.g. high water retention, high nutrient content).

4.2. Seasonal distribution

The seasonal fluctuations in chlorophyll a content and primary production (Sundbäck, 1983) were not reflected in blooms of single diatom species. However, irregular variation was observed from one sampling occasion to another especially at station 2. Although the variation may be partially related to differences in sampling and counting strategy, on two occasions a chlorophyll a peak coincided with a peak in the relative abundance of *Opephora martyi*. However, values for relative abundance should be interpreted with caution (cf. Thomas, 1979). Moreover, a slight increase in numbers of a large species (e.g. *Navicula phyllepta*) has the same effect on the total biomass as a large increase in numbers of a very small species (e.g. *Fragilaria pinnata*; see Table 10).

Why is the lack of marked seasonal variation so apparent in the sediments investigated here, when seasonal fluctuations have been demonstrated for benthic diatoms on tidal flats, for instance (van den Hoek et al., 1979; Riaux and Germain, 1980; Rincé et al. 1981). Colijn and Dijkema (1981), however, separated the diatoms into species with and without temporal variations. They mention *Achnanthes hauckiana*, which is one of the most stable species both in Nivå Bay (M. Gargas, 1980) and in my investigation, as a species with temporal variation. Van den Hoek et al. (1979), suggest that the seasonal behaviour

of a species may differ with place. They also suggest that the bulk of the species composition of benthic diatoms in the Wadden Sea consists of species that are present all year round.

The homogeneity of the species composition was especially pronounced at station 1. Colijn and Dijkema (1981) found that 'cluster stability' was related to uniformity of the composition of the sediment at the stations throughout the year. It has been suggested that uniformity due to lack of stability of the sediment in connection with constant availability of nutrients accounts for both the spatial and temporal homogeneity of the sediment flora (Amspoker, 1977; Amspoker and McIntire, 1978; Hunding, 1971).

The structural stability of the diatom flora of my sampling stations was combined with stable and high degree of diversity and evenness. High diversity values have also been found by others for benthic diatom communities (e.g. Amspoker, 1977; Amspoker and McIntire, 1978). This has been considered a surprising feature of the intertidal habitat, which is regarded as being a fluctuating and stressful habitat. Heavy grazing (Amspoker, 1977) and the dynamic (though not stressful) nature of the habitat which creates numerous microniches (Sullivan, 1978) have been suggested as possible causes.

Numerical analysis indicated a slight change in the structure of the diatom community from 1977 to 1980. At station 2 this could have been caused by a slight change in sampling position. At station 1, however, sampling strategy alone can hardly serve to explain the difference since the sampling site is restricted and the sand homogeneous owing to the action of swell. Errors due to inconsequent identification should also be excluded, since all material from Period I was revised in 1980. Thus we cannot completely overlook the possibility of year-to-year variation in the composition of benthic diatom communities. For instance very little is known about interactions between diatoms and other organisms (e.g. the year-to-year fluctuation of fauna and macrovegetation).

My results, which indicate a high degree of diversity and fairly stable species composition of the benthic diatom flora, support the following hypothesis, which agrees with Amspoker

(1977) and Amspoker and McIntire (1978):

The microphytobenthic diatom flora of sandy bottoms in semi-exposed shallow waters investigated here, is dominated by well-adapted epipsammic diatoms. Since the life forms are not capable of rapid phototactic or chemotactic movement (but note Harper , 1969), as are the epipelagic diatoms of the tidal flats, the existence of the algae requires adaptation to the habitat. This adaptation involves high photosynthetic capacity in varying light intensities (Hunding, 1971; Gargas, 1971; Rasmussen et al., 1983), tolerance of prolonged burial under aphotic conditions (Moss, 1977; Gargas and Gargas, 1982a, b), and strong frustules and a well-developed ability to adhere to sand grains (Harper and Harper, 1967) to withstand the mechanical effects of water movement. In addition the habitat is rich in nutrients and the sediment may reduce the effect of fluctuating physical factors (cf. Amspoker and McIntire, 1978). Thus there seem to be no typical winter or summer species the flora comprising the same epipsammic species all year round. When the biomass increases with rising temperatures in spring (Sundbäck, 1983), there is an increase in biomass of all species. In this area rough seas are uncommon in spring, summer and early autumn (von Wachenfeldt, 1975) and the motile and loosely attached life forms are also able to increase their biomass. This is reflected in the larger proportion of species of life forms (b) and (c) found here during these seasons (Fig. 4). Similarly the measurements of ^{14}C -uptake by the suspendible fraction were highest during early spring, late summer and autumn (Sundbäck, 1983). Thus, as with spatial distribution, an important factor regulating temporal distribution may be mechanical disturbance by water movement.

4.3. Can glass be used to study the structure of diatom assemblages on sandy bottoms?

Here glass was used in the colonization experiment, since it has been often used in periphyton research and since it enables direct examination under the microscope.

The glass slides, even though they were pressed into the sediment, provided a substrate that favoured the development of an epiphytic rather than of an epipsammic flora. In comparison with the natural sediment flora, the diatom community of the glass slides was characterized by a lower degree and more fluctuating diversity and lower dimensionality owing to the invasion of *Cocconeis placentula* (cf. DeFelice and Lynts, 1978, who also observed a low degree of diversity for the epiphytic *Cocconeis placentula* association as compared with epipellic associations in Florida Bay).

The epiphytic nature of the assemblage was also illustrated by the presence of young stages of macroalgae and large protruding diatoms such as *Licmophora* spp., which are hardly ever found in the sediment at this sampling site.

Artificial substrates are frequently used for periphyton studies, especially water quality studies (for review see Weitzel, 1979). Several comparative studies suggest, that the communities that develop on artificial substrates do not reflect the structure of the community of natural substrates (Tippet, 1970; Brown, 1976; Siver, 1977). These studies, however, deal with epiphytic or epilithic communities and not with diatoms inhabiting sediments. McIntire and Overton (1971) and Moore and McIntire (1977) used PVC plates to study the distribution of attached diatoms on estuary. However, in the latter article the authors report that the distributional trends revealed by the diatom communities developing on artificial substrates probably did not apply to the diatom flora of the sediments. This was shown to be true by Amspoker and McIntire (1978). It can thus be concluded that the conventional colonizing methods at least, where the substrates are usually suspended in the water are not applicable to in situ studies of sediment diatoms.

The close resemblance between the diatom flora of the glass slides and natural substrate during the initial stages when colonization proceeded slowly suggests that if colonization is interrupted at a certain stage close resemblance to the natural assemblage is attained. Moore and McIntire (1977) present a structural model of the colonization process and the many mechanisms involved. Colonization is a dynamic process dependent on among other things, the species pool, length of time and interactions between species. Little is known about either interspecific interactions within diatom populations or the effect of fortuitous events on taxonomic compositions and dominance (Moore and McIntire, 1977).

The colonization experiment suggests that the smooth and stationary glass slides are a poor imitation of the natural habitat (sand grains) with its microniches. Thus the dominants of the sediment flora were replaced by species that can adapt to living on a smooth artificial substrate. However, the use of artificial substrates may, with appropriate modifications, be practicable in laboratory experiments, e.g. for studying interaction between diatom species.

4.4. Methodological remarks

An accurate description of the microphytobenthic community from diatom counts requires that (1) diatoms are the main biologically important constituents of the microflora and (2) the relative abundance of cleaned diatom cells do not significantly affect the results. The first prerequisite is based on semi-quantitative (subjective) examination of fresh collections (see 3.1.). I considered that the dominance of diatoms in the microflora (80--90%) justified restricting further discussion to diatoms only. However, when discussing seasonal variation it must be kept in mind that other autotrophic organisms may occur, especially at station 2, and that these organisms may have another type of seasonal behaviour than benthic diatoms.

The second prerequisite involves two problems. Firstly, the use of proportions instead of cell densities may bias the interpretation of the statistical analyses (Thomas, 1979). The use of cell densities was not felt necessary in planning this investigation, since other community parameters such as chlorophyll a and ^{14}C -uptake were used (Sundbäck, 1983). Secondly, the difficulty of distinguishing between living and dead diatom cells may affect the results. The conventional method involving cleaned diatom suspensions also used by ecologists (e.g. McIntire and Overton, 1971; McIntire, 1973; Amspoker, 1977; Colijn and Nienhuis, 1978; DeFelice and Lynts, 1978) is justified by the fact that certain identifications can be made only on oxidized cells. Several methods for separating living and dead diatom cells have been presented, e.g. using the fluorescent microscope, or making permanent slides of living or stained diatom cells (Owen et al., 1979; Thomas, 1979). The users of these methods, however, admit that problems arise for species identification, e.g. for the genus *Navicula* (Thomas, 1979). Since most diatom cells in this investigation are small and densely striated, the above methods could hardly be used. Recently de Jonge (1979) succeeded in developing a method where living diatoms can be separated from empty frustules and sediment particles by density gradient centrifugation (the Ludox method).

The proportions of dead cells reported from sediment samples vary between c. 10% (Colijn et al., 1976) and 25% (Taasen and Høisaeter, 1981). According to Owen et al. (1979) the proportion of dead cells in periphyton is at least 10% (range 1--80%) and seems to vary with the season. The number of dead diatom cells may also vary along the exposure-gradient (van den Hoek et al., 1979), in which case more dead frustules should occur at station 2 than at station 1.

Table 3 suggests that as far as relative abundance can reflect the true composition of the diatom community (see Thomas, 1979), the inclusion of dead cells is probably not a significant artifact in the Falsterbo material. Thomas (1979) found that the proportions of cleaned cells were much the same as for living cells. The Shannon index H' , used here for diversity, does not either appear to be greatly affected by the inclusion of dead cells (Owen et al., 1979).

4.5. Problems of identification

The identification of the diatom species was often problematic. Some taxa were not found in the standard taxonomic literature and some specimens did not completely fit the description of a species (see also Sundbäck, 1982).

The commonest *Opephora* species varied greatly from small subspherical to long narrow specimens (Plate 1:d, Plate 2:c,f,i). I included most of these forms in *O. martyi* Héribaud because of the characteristic coarse striation and the narrow pseudoraphe. *O. olsenii* Möller may also be present. According to Möller (1950) *O. olsenii* is distinguished from *O. martyi* on the narrower cells and the distinct ring of spicules on the valvar edge. The latter character was difficult to distinguish under the light microscope. SEM micrographs, however, indicate that two different forms existed in the material. Extremely small specimens of *Opephora* with delicate striation were placed in a separate unidentified taxon, *O. sp. 1*.

Achnanthes hauckiana Grunow varied widely in both size and shape. The smallest specimens were nearly round, but in spite of their size (down to 5.5 μ m length), they displayed the characteristic coarse and conical striae. I made no attempt to separate varieties, since there were intermediate forms (cf. Tropper, 1975). Based on 245 measured cells from different localities in Öresund *A. hauckiana* varied from 5.5 to 17 μ m in length, 3.4 to 7.1 μ m in breadth and had 9--16 striae in 10 μ m (Sundbäck, unpublished).

One of the commonest *Achnanthes* species was not identified with certainty (length 7.5--9.5 μ m, breadth c. 3.5 μ m, striae 16.5--18/10 μ m; Plate 2:b,h). However, it resembles *A. tenera* Hustedt. The one shorter stria shown in Hustedt's figure (Plate 5:22-25, 1955) is also characteristic of most specimens found in the Falsterbo area, although the shape of the valve ends varied from rounded to acute subrostrate.

The *Cocconeis placentula* Ehrenberg were mainly small (length c. 10 μ m) and var. *euglypta* (Ehrenberg) Cleve, but the distinction between different varieties was not clear, and all forms were combined to a single taxonomic unit, *C. placentula* et var.

Cocconeis sp. 1 resembles *C. peltoides* Hustedt but the longitudinal lines crossing the striation were not distinct (Plate 2: e). When measuring *C. peltoides* cells from different salinity ranges in Öresund, there was a tendency for typical *C. peltoides* to be replaced by the non-typical in lower salinity.

Navicula sp. 1 is easily recognized by the clearly lineolate striation, the slightly curved raphe and the distant central nodules (Plate 2: d, 2: g). Cell length is fairly constant, c. 10 μ m. A similar *Navicula* sp. has been reported by M. Gargas (1980) from Nivå Bay on the Danish coast of Öresund.

Navicula sp. 5 can be described as resembling a minute (<10 μ m long) densely striated, slightly rostrate *Navicula salinarum* Grunow (Plate 2: a). Small specimens of *N. cryptocephala* Kützing and *N. salinarum* can easily be confused with *N.* sp. 5 and it is possible that *N.* sp. 5 is one of these two species, both of which are common in the western Baltic (e.g. Simonsen, 1962).

The genus *Amphora* Ehrenberg involved many taxonomic problems. However, in spite of its smallness the commonest species could be identified as *Amphora exigua* Gregory on the eight striated divisions in girdle view (Hendey, 1964). In addition to *A. exigua*, *A. coffeaeformis* (Agardh) Kützing (small forms) and *A. tenerrima* Aleem & Hustedt were found and could be confused. Small hyaline species of *Amphora* were common, e.g. *A. abludens* Simonsen and *A.* sp. 1, the latter being extremely hyaline and narrow and with no discernible striation under the light microscope.

The explanation of the deviation in morphology of benthic diatoms in the Falsterbo area, that lies closest to hand is low salinity. The horizontal salinity gradient of Öresund is reflected in the morphology of macroalgae (von Wachenfeldt, 1975). Using culture experiments Geissler (1970) has demonstrated the effect of salinity on the morphology of diatoms. Simonsen (1962) also mentions the variation in morphology of *Navicula salinarum* with salinity. A comparison of the size of some benthic diatoms from different parts of Öresund (Sundbäck, unpublished) indicates that marine species become smaller southwards with decreasing salinity but further sampling and controlled culture experiments are necessary.

The lower limit of survival for marine benthic diatoms has been put at salinity around 5 ‰ (see Admiraal, 1977b; Amspoker and McIntire, 1978; van den Hoek et al., 1979). The average salinity in the Falsterbo area is somewhat higher, 8--9 ‰. One important difference, however, between a tidal area and the Falsterbo area, is the lack of tide-induced regular and strong salinity fluctuations. The ability of benthic diatoms to tolerate large ranges of salinity fluctuations may thus be less in Öresund and the Baltic.

That there are taxonomic problems in connection with benthic brackish-water diatoms is also evident from other areas as seen by the many unidentified taxa in species lists (e.g. McIntire and Overton, 1971; Colijn and Nienhuis, 1978; Amspoker, 1977). Amspoker and McIntire (1978) report that more than 30% of the total number of cells counted from Yaquina Estuary sediment could not be identified. It is probable that many small diatom species have been previously overlooked and have thus remained undescribed. Since diatom species tend to have a worldwide distribution (McIntire and Moore, 1977) it is probable that many taxa reported as unidentified from sediment from different parts of world may be the same species. This underlines the necessity of illustrating unidentified species even in ecological papers. Since the value of an ecological microphytobenthic investigation that includes information on species, is dependent on the accurate identification of specimens, it is of extreme importance that taxonomic investigations on the benthic diatoms, especially the epipsammon should be encouraged.

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Plate 1. SEM micrographs (ISI M7) of air-dried sand grains: (a) Sand grain with diatoms in the cavities, (b) detail of (a), (c) Navicula and Achnanthes (life form (a)) in a cavity of a sand grain, (d) Opephora colony (life form (b)) on a sand grain. Scale, 100 μm in a and 10 μm in b-d. Photos by H. Håkansson and K. Sundbäck.

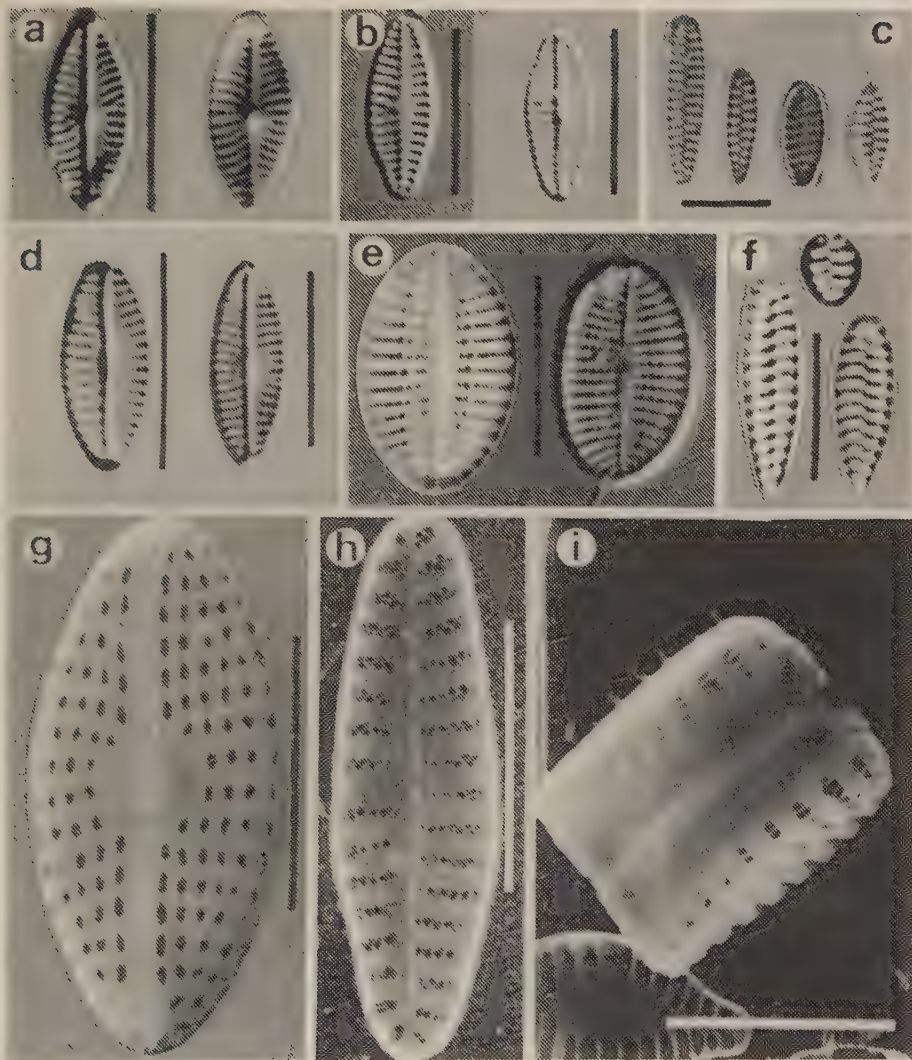


Plate 2. LM micrographes a-f: (a) Navicula sp. 5, (b) Achnanthes cf. tenera Hustedt, (c) Opephora martyi Héribaud incl. O. olsenii Möller, (d) Navicula sp. 1, (e) Cocconeis sp. 1, (f) Opephora martyi Héribaud; SEM micrographs g-i: (g) Navicula sp. 1, (h) Achnanthes cf. tenera Hustedt, (i) Opephora cf. martyi Héribaud. Scale, 10 μ m in a-f and i, and 5 μ m in g and h.

APPENDIX 1

Dates of sampling occasions included in the numerical analyses

Date	Station 1 Sediment	Station 2 Sediment	Station 2 Glass slides
Period I			
1975-10-23	x	x	
1976-01-23	x	x	
03-08		x	
04-07	x		
09-23	x	x	
10-07	x	x	
1977-03-03	x	x	
04-08		x	
05-14	x	x	
05-28	x	x	
06-25	x	x	
07-29	x	x	
08-20	x	x	
09-04	x	x	
09-29	x	x	
10-20	x	x	
11-17	x		
1978-01-31	x	x	
04-08	x	x	
Period II			
1980-04-06	x	x	
05-18	x	x	x
06-01	x	x	x
06-29	x	x	x
07-27	x	x	x
08-24	x	x	x
09-14	x	x	x
10-19	x	x	x
11-16	x	x	x
Total number of days:	26	26	8

APPENDIX 2

Species list and abbreviations of the species names. Taxa in brackets are not included in the numerical analysis.

<u>Species</u>	<u>Abbreviation</u>
<i>Achnanthes biasoletiana</i> (Kützing) Grunow	Ac bias
<i>A. brevipes</i> Agardh	Ac brev
<i>A. cf. conspicua</i> Mayer	Ac cons
<i>A. delicatissima</i> Simonsen	Ac delis
<i>A. delicatula</i> (Kützing) Grunow	Ac delic
<i>A. exigua</i> Grunow	Ac exig
<i>A. hauckiana</i> Grunow	Ac hauc
<i>A. lemmermannii</i> Hustedt	Ac lemm
<i>A. cf. levanderii</i> Hustedt	Ac leva
<i>A. punctulata</i> Simonsen	Ac punc
<i>A. pseudopunctulata</i> Simonsen	Ac pspu
<i>A. pseudosolea</i> Simonsen	Ac psso
<i>A. cf. tenera</i> Hustedt	Ac tene
<i>A. sp. 1</i>	Ac sp 1
<i>Amphora abludens</i> Simonsen	Am ablu
<i>A. coffeaeformis</i> (Agardh) Kützing	Am coff
<i>A. cf. coffeaeformis</i> var. <i>perpusilla</i> Grunow	Am coff perp
<i>A. exigua</i> Gregory	Am exig
<i>A. holsatica</i> Hustedt	Am hols
<i>(A. laevis</i> var. <i>laevissima</i> (Gregory) Cleve)	
<i>A. lineolata</i> Ehrenberg	Am line
<i>A. ostrearia</i> Brébisson	Am ostr
<i>A. ovalis</i> var. <i>libyca</i> (Ehrenberg) Cleve	Am oval liby
<i>A. ovalis</i> var. <i>pediculus</i> (Kützing) van Heurck	Am oval pedi
<i>A. tenerrima</i> Aleem & Hustedt	Am tene
<i>(A. turgida</i> Gregory)	
<i>A. veneta</i> Kützing	Am vene
<i>A. sp. 1</i>	Am sp 1
<i>A. sp. 2</i>	Am sp 2
<i>A. sp. 3</i>	Am sp 3
<i>(Amphiprora alata</i> (Ehrenberg) Kützing)	
<i>(Berkeleya rutilans</i> (Trentepohl) Grunow)	
<i>Caloneis amphisbaena</i> (Bory) Cleve	Ca amph
<i>Catenula adhaerens</i> Mereschkowsky	Ct adha
<i>Cocconeis diminuta</i> Pantocsek	Co dimi
<i>(C. pediculus</i> Ehrenberg)	
<i>C. peltoides</i> Hustedt	Co pelt
<i>C. placentula</i> et var. Ehrenberg	Co plac
<i>C. scutellum</i> Ehrenberg	Co scut
<i>C. scutellum</i> var. <i>stauroneiformis</i> Rabenhorst	Co stau
<i>C. sp. 1</i>	Co sp 1
<i>C. sp. 2</i>	Co sp 2
<i>(Cylindrotheca closterium</i> Reimann & Lewin)	
<i>(Diatoma elongatum</i> (Lyngbye) Agardh)	
<i>D. elongatum</i> var. <i>tenuis</i> (Agardh) Van Heurck	Di elon tenu
<i>Diploneis didyma</i> (Ehrenberg) Ehrenberg	Dp didy
<i>(D. interrumpita</i> (Kützing) Cleve)	
<i>(D. cf. puella</i> (Schumann) Cleve)	
<i>D. smithii</i> (Brébisson) Cleve	Dp smit

(<i>Epithemia argus</i> (Ehrenberg) Kützing)	
<i>E. sorex</i> Kützing	Ep sore
<i>E. turgida</i> (Ehrenberg) Kützing.	Ep turg
(<i>E. zebra</i> (Ehrenberg) Kützing)	
(<i>Fragilaria bicapitata</i> Mayer)	
<i>F. construens</i> var. <i>subsalina</i> Hustedt	Fr cons subs
<i>F. construens</i> var. <i>venter</i> (Ehrenberg) Grunow	Fr cons vent
<i>F. pinnata</i> Ehrenberg	Fr pinn
<i>F. virescens</i> var. <i>subsalina</i> Grunow	Fr vir subs
<i>F. sp. 1</i>	Fr sp 1
<i>Gomphonema abbreviatum</i> (Agardh) Kützing	Go abbr
(<i>G. olivaceum</i> (Lyngbye) Kützing)	
<i>G. pseudoerigium</i> Simonsen	Go pseu
<i>Grammatophora marina</i> (Lyngbye) Kützing	Gr mari
<i>G. oceanica</i> (Ehrenberg) Grunow	Gr ocea
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	Gy acum
<i>Hantzschia virgata</i> (Roper) Grunow	Ha virg
<i>Licmophora abbreviata</i> Agardh	Li abbr
<i>L. gracilis</i> (Ehrenberg) Grunow	Li grac
<i>L. rhombica</i> Möller	Li rhom
<i>Mastogloia elliptica</i> (Agardh) Cleve	Ma elli
<i>M. exigua</i> Lewis	Ma exig
<i>M. pumila</i> (Grunow) Cleve	Ma pumi
<i>Navicula aberrans</i> Simonsen	Na aber
<i>N. abbreviata</i> (Grunow) Cleve-Euler	Na abbr
<i>N. ammophila</i> Grunow	Na ammo
<i>N. amphipleuroides</i> Hustedt	Na amphi
<i>N. arenicola</i> Grunow	Na arni
<i>N. bahusiensis</i> Grunow	Na bahu
<i>N. biskanteri</i> Hustedt	Na bisk
<i>N. cancellata</i> Donkin	Na canc
<i>N. cincta</i> (Ehrenberg) Ralfs	Na cinc
<i>N. clamans</i> Hustedt	Na clam
<i>N. clementis</i> Grunow	Na clem
<i>N. crucicula</i> (W Smith) Donkin	Na crula
<i>N. cruciloides</i> Brockmann	Na crudes
<i>N. crucifera</i> Grunow	Na crufe
<i>N. cryptocephala</i> et var. Kützing	Na cryce
<i>N. cryptolyra</i> Brockmann	Na cryly
<i>N. digitoradiata</i> (Gregory) Ralfs	Na digi
<i>N. diserta</i> Hustedt	Na dise
<i>N. fenestrella</i> Hustedt	Na fene
<i>N. flantica</i> Grunow	Na flana
<i>N. florinae</i> Möller	Na flor
<i>N. forcipata</i> Greville	Na forc
<i>N. gracilis</i> Ehrenberg	Na grac
(<i>N. grevillei</i> (Agardh) Heiberg)	
<i>N. hanseni</i> Möller	Na hans
<i>N. cf. incerta</i> Grunow	Na incer
<i>N. menisculus</i> Schumann	Na melu
<i>N. nolens</i> Simonsen	Na nole
<i>N. peregrina</i> (Ehrenberg) Kützing	Na pere
<i>N. peregrina</i> var. <i>meniscus</i> (Schumann) Grunow	Na pere meni
<i>N. phyllepta</i> Kützing	Na phyll
<i>N. rhyncocephala</i> Kützing	Na rhyn
<i>N. salinarum</i> et var. Grunow	Na sali
<i>N. cf. salinicola</i> Hustedt	Na salco

<i>Navicula subinflata</i> Grunow	Na subi
<i>N. teneroides</i> Hustedt	Na tene
<i>N. viridula</i> (Kützing) Ehrenberg	Na viri
<i>N. sp. 1</i>	Na sp 1
<i>N. sp. 2</i>	Na sp 2
<i>N. sp. 3</i>	Na sp 3
<i>N. sp. 4</i>	Na sp 4
<i>N. sp. 5</i>	Na sp 5
<i>N. sp. 6</i>	Na sp 6
<i>Nitzschia amphibia</i> Grunow	Ni amph
(<i>N. communis</i> Grunow)	
(<i>N. constricta</i> (Kützing) Ralfs)	
<i>N. dissipata</i> (Kützing) Grunow	Ni diss
<i>N. frustulum</i> var. <i>subsalina</i> Hustedt	Ni frus subs
(<i>N. hybrida</i> Grunow)	
<i>N. kuetzingiana</i> Hilse	Ni kuetz
<i>N. kuetzingiana</i> var. <i>exilis</i> Grunow	Ni kuetz exil
<i>N. microcephala</i> Grunow	Ni micro
<i>N. sigma</i> (Kützing) Wm. Smith	Ni sigm
<i>N. tryblionella</i> var. <i>levidensis</i> (Wm. Smith) Grunow	Ni tryb levi
<i>N. valdestriata</i> Aleem & Hustedt	Ni vald
<i>N. sp. 1</i>	Ni sp 1
<i>Opephora marina</i> (Gregory) Petit	Op mari
<i>O. martyi</i> Hérivaud	Op mart
<i>O. schulzii</i> Brockmann	Op schu
<i>O. sp. 1</i>	Op sp 1
<i>Pinnularia ambigua</i> Cleve	Pi ambi
(<i>P. globiceps</i> Gregory)	
(<i>P. globiceps</i> var. <i>krockii</i> (Grunow) Cleve)	
(<i>P. quadratarea</i> (Smidt) Cleve)	
<i>Pleurosigma angulatum</i> (Quekett) Wm. Smith	Pl angu
<i>Rhoicosphenia curvata</i> (Kützing) Grunow	Rh curv
(<i>Rhopalodia gibberula</i> et var. (Ehrenberg) Müller)	
<i>Staroneis salina</i> Wm. Smith	St sali
<i>Synedra tabulata</i> (Agardh) Kützing	Sy tabu
<i>S. tabulata</i> var. <i>acuminata</i> (Grunow) Hustedt	Sy tabu acum
<i>S. tabulata</i> var. <i>fasciculata</i> (Lyngbye) Hustedt	Sy tabu fasc
<i>S. ulna</i> (Nitzsch) Ehrenberg	Sy ulna
<i>Tropidoneis lepidoptera</i> (Gregory) Cleve	Tr lepi

REFERENCES

- Admiraal, W. 1977a: Influence of light and temperature on the growth rate of estuarine diatoms in culture.- *Mar. Biol.* 39:1-11.
- Admiraal, W. 1977b: Salinity tolerance of benthic diatoms as tested with a rapid polarographic measurement of photosynthesis.- *Mar. Biol.* 39:11-18.
- Admiraal, W. 1980: Experiments on the ecology of benthic diatoms in the Eems-Dollard estuary.- *Doct. thesis, BOEDE publ. No. 3.*
- Admiraal, W. and Peletier, H. 1979: Sulphide tolerance of benthic diatoms in relation to their distribution in an estuary.- *Br. phycol. J.* 14: 185-196.
- Admiraal, W. and Peletier, H. 1980: Distribution on diatom species on an estuarine mudflat and experimental analysis on the selective effect of stress.- *J. exp. mar. Biol. Ecol.* 46:157-175
- Admiraal, W., Peletier, H. and Zomer, H. 1982: Observations and experiments on the population dynamics of epipellic diatoms from an estuarine mudflat.- *Est. Coast. Shelf. Sc.* 14:471-487.
- Amspoker, M.C. 1977: The distribution of intertidal epipsammic diatoms on Scripps Beach, La Jolla, California, USA.- *Bot. Mar.* 20:227-232.
- Amspoker, M.C. and McIntire C.D. 1978: Distribution of intertidal diatoms associated with sediments in Yaquina Estuary, Oregon.- *J. Phycol.* 14: 387-395
- Brockmann, G. 1950: Die Watt-Diatomeen der Schleswig-Holsteinischen Westküste.- *Abh. seckenb. naturf. Ges.* 478:1-26
- Brown, H.D. 1976: A comparison of attached algal communities of a natural and an artificial substrate.- *J. Phycol.* 12:301-306.
- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea.- *Neth. J. Sea Res.* 8:260-291.
- Cleve-Euler, A. 1951-1955: Die Diatomeen von Schweden und Finnland.- *K. Sv. Vetensk. Akad. Handl. Ser. 4: I-V*, 959 pp., Stockholm.
- Colijn, F. and Koeman, R. 1975: Das Mikrophytobenthos der Watten, Strände und Riffe um den Hohen Knechtsand in der Wesermündung.- *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 26:53-83.
- Colijn, F., Nienhuis, H., de Jonge, V.N. and Koeman, R. 1976: Distribution and seasonal periodicity of sediment inhabiting benthic diatoms in the Wadden Sea and the Ems-Dollard estuary.- *Jaarboek 1975 Kon. Ned. Bot. Ver.*, 3 pp.
- Colijn, F. and Nienhuis, H. 1978: The intertidal microphytobenthos of the 'Hohe Weg' shallows in the German Wadden Sea.- *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 29:149-174.
- Colijn, F. and Dijkema, K.S. 1981: Species composition of benthic diatoms and distribution of chlorophyll *a* on an intertidal flat in the Dutch Wadden Sea.- *Mar. Ecol.* 4:9-21.
- Cook, L.L. and Whipple, S.A. 1982: The distribution of edaphic diatoms along environmental gradients of a Louisiana salt marsh.- *J. Phycol.* 18:64-21.
- DeFelice, D.R. and Lynts, G.W. 1978: Benthic marine diatom associations: Upper Florida Bay (Florida) and associated sounds.- *J. Phycol.* 14:25-33.
- Dickman, M. and Gochbauer, M.B. 1978: A scanning electron microscope study on periphyton colonization in a small stream subjected to sodium chloride addition.- *Verh. Internat. Verein. Limnol.* 20:1738-1743.
- Edler, L. (ed.) 1979: Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll.- *BMB Publ. No. 5.*
- Gargas, E. 1970: Measurements of primary production, dark fixation and vertical distribution of microbenthic algae in the Öresund.- *Ophelia* 8:231-253.
- Gargas, E. 1971: 'Sun-shade' adaptation in microbenthic algae from the Öresund.- *Ophelia* 9:107-112.

- Gargas, M. 1980: Ökofysiologi og artsammansættning hos mikrobenthiske diatomeer i Nivå bugt.- M.Sc. thesis, 384 pp. University of Copenhagen.
- Gargas, M. and Gargas, E. 1982a: Growth physiological conditions of marine microalgae in the topmost 10 cm of the sediment.- *Vatten* 38:189-198.
- Gargas, M. and Gargas, E. 1982b: Influence of temperature and oxygen conditions on phytomicrobenthos stored for longer periods in darkness.- *Vatten* 38:306-316.
- Geissler, U. 1970: Die Variabilität der Schalenmerkmale bei den Diatomeen.- *Nova Hedwigia* 19:623-773.
- Grøntved, J. 1960: On the productivity of microphytobenthos and phytoplankton in some Danish fjords.- *Medd. Danm. Fiskeri- og Havsunders.* 3:55-92.
- Haardt, H. and Nielsen, G.A. 1980: Attenuation measurements of monochromatic light in marine sediments.- *Oceanologica Acta* 3:333-338.
- Harper, M.A. 1969: Movement and migration of diatoms on sand grains.- *Br. phycol. J.* 4:97-103.
- Harper, M.A. and Harper, J.F. 1967: Measurements of diatom adhesion and their relationship with movement.- *Br. phycol. Bull.* 3:195-207.
- Hendey, N.I. 1964: An introductory account of the smaller algae of the British coastal waters. Part V: Bacillariophyceae (Diatoms).- 317 pp. London.
- Hoek, van den C., Admiraal, W., Colijn, F. and de Jonge, V.N. 1979: The role of algae and seagrasses in the ecosystem of the Wadden Sea: A review.- In Wolff, W.J. (ed.) *Flora and vegetation of the Wadden Sea*: 3:1-206. Rotterdam.
- Hunding, C. 1971: Production of benthic microalgae in the littoral zone of an eutrophic lake.- *Oikos* 22:389-397.
- Hustedt, F. 1930: Bacillariophyta (Diatomae). In Pascher, A. (ed.) *Süßwasserflora Mitteleuropas*. 466 pp. Jena.
- Hustedt, F. 1930-1966: Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete.- In Rabenhorst, L. (ed.) *Kryptogamenflora* 7. 2581 pp. Leipzig.
- Hustedt, F. 1939: Die Diatomeenflora des Küstengebietes der Nordsee vom Dollart bis zur Elbemündung.- *Abh. hrsg. naturwiss. Ver. Bremen* 31: 572-677.
- Hustedt, F. 1955: Marine littoral diatoms of Beaufort, North Carolina.- *Duke Univ. Marine Station Bull.* 6:1-67.
- Håkansson, H. and Stabell, B. 1977: Identification of some small species of *Navicula*.- *Bot. Not.* 130:477-481.
- Jansson, A-M., Kautsky, N., von Oertzen, J-A., Schramm, W., Sjöstedt, B., von Wachenfeldt, T. and Wallentinus, I. 1982: Structural and functional relationships in a southern Baltic *Fucus* ecosystem. A joint study by the BMB phytobenthos group. *BMB Publ.* No. 8.
- Jonge, de V.N. 1979: Quantitative separation of benthic diatoms from sediments using density gradient centrifugation in the colloidal silica Ludox-TM.- *Mar. Biol.* 51:267-278.
- Jonge, de V.N. (in press): The occurrence of epipsammic diatom populations: a result of interaction between physical sorting of sediment and some properties of diatom species.- *Est. Coast. Shelf. Sci.*
- Lange-Bertalot, H. 1977: Eine revision zur Taxonomie der Nitzschiae lanceolatae Grunow.- *Nova Hedwigia* 28:253-307.
- Lange-Bertalot, H. 1980: Zur taxonomischen Revision einiger ökologisch wichtiger 'Naviculae lineolatae' Cleve.- *Cryptogamie, Algologie* 1:29-50.
- Lange-Bertalot, H. and Ruppel, M. 1980: Zur Revision taxonomisch, ökologisch, jedoch wichtiger Sippen der Gattung *Achnanthes* Bory.- *Arch. Hydrobiol./Suppl.* 60. *Algalogical Studies* 26:1-31.
- Liljelund, L-E. 1977: Diversitetsindex - en översikt.- *Svensk Bot. Tidskr.* 71:165-176.

- Maarel, van der, E., Janssen, J.G.M. and Louppen, J.M.V. 1978: TABORD, a program for structuring phytosociological tables.- *Vegetatio* 38:143-156.
- McIntire, C.D. 1973: Diatom associations in Yaquina Estuary, Oregon: A multivariate analysis.- *J. Phycol.* 9:254-259.
- McIntire, C.D. 1978: The distribution of estuarine diatoms along environmental gradients: A canonical correlation.- *Est. Coast. Mar. Sc.* 6: 447-459.
- McIntire, C.D. and Overton, W.S. 1971: Distributional patterns in assemblages of attached diatoms from Yaquina Estuary, Oregon.- *Ecology* 52:758-777.
- McIntire, C.D. and Moore, W.W. 1977: Marine littoral diatoms - Ecological considerations.- In Werner, D. (ed.) *The biology of diatoms*: 333-371. Oxford.
- Moore, W.W. and McIntire, C.D. 1977: Spatial and seasonal distribution of littoral diatoms in Yaquina Estuary, Oregon (USA).- *Bot. Mar.* 20:99-109.
- Moss, B. 1977: Adaptations of epipellic and epipsammic freshwater algae.- *Oecologia* 28: 103-108.
- Moss, B. and Round, F.E. 1967: Observations on standing crops of epipellic and epipsammic algal communities in Shear Water, Wilts.- *Br. phycol. Bull.* 3: 241-248.
- Möller, M. 1950: The diatoms of Prästö Fjord.- *Folia Geogr. Danica* 5:187-237.
- Owen, B.B., Afzal, M. and Cody, W.R. 1979: Distinguishing between live and dead diatoms in periphyton communities.- In Weitzel, R.L. (ed.) *Methods and measurements of periphyton communities: A review*, pp. 70-76. Philadelphia.
- Pamatmat, M.M. 1968: Ecology and metabolism of a benthic community on an intertidal sandflat.- *Int. Rev. ges. Hydrobiol.* 53:211-298.
- Pankow, H. 1976: *Algenflora der Ostsee. II. Plankton*. 493 pp. Jena.
- Pankow, H. 1980: Die benthischen Kieselalpengesellschaften der Boddengewässer des Dars und des Zingst (Südliche Ostsee).- *Wiss. Zeitschr. Wilhelm-Pieck-Univ. Rostock* 29:131-137.
- Patrick, R. and Reimer, C.W. 1966: *The diatoms of the United States*. 688 pp. Philadelphia.
- Persson, S. 1977: Datorprogram för bearbetning av vegetationsdata. 1. Klassifikationsprogram - dokumentation och handhavande.- *Meddn Avd. Ekol. Bot., Lunds Universitet* 33, 68 pp. Lund.
- Persson, S. 1978: Datorprogram för bearbetning av vegetationsdata. 2. Ordinationsprogram - dokumentation och handhavande.- *Meddn Avd. Ekol. Bot., Lunds Universitet* 34, 64 pp. Lund.
- Pielou, E.C. 1966: Species-diversity and pattern-diversity in the study of ecological succession.- *J. Theor. Biol.* 10:370-383
- Pomeroy, L.R. 1959: Algal productivity in salt marshes of Georgia.- *Limnol. Oceanogr.* 4:386-397.
- Rao, V.N.R. and Lewin, J. 1976: Benthic marine diatom flora of False Bay, San Juan Island, Washington.- *Syesis* 9:173-213.
- Rasmussen, M.B., Henriksen, K. and Jensen, A. 1983: Possible causes of temporal fluctuations in primary production of the microphytobenthos in the Danish Wadden Sea.- *Mar. Biol.* 73:109-114.
- Riaux, C. and Germain, H. 1980: Peuplement de diatomées épipéliques d'une slikke de Bretagne Nord. Importance relative de genre *Cocconeis* Ehr.- *Cryptogamie, Algologie* 1:265-279.
- Rincé, Y., Plante-Cuny, M.-R., Riaux, C., Robert, J.-M. and Malissen, M.-O. 1981: Comparison between benthic diatom populations in muddy sediments of four localities along the French western coast.- In Ross, R. (ed.) *Proceedings of the Sixth Symposium on recent and fossil diatoms*: pp. 371-384.
- Roskam, E. 1971: Program ORDINA: Multidimensional ordination of observation vectors.- *Programme Bull.* 16., Dept of Psychology, University of Nijmegen. 8 pp.

- Round, F.E. 1965: The epipsammon; a relatively unknown freshwater algal association.- Br. phycol. Bull. 2:456-462.
- Round, F.E. 1971: Benthic marine diatoms.- Oceanogr. Mar. Biol. Ann. Rev. 9:83-139.
- Round, F.E. 1979: A diatom assemblage living below the surface of intertidal sand flats.- Mar. Ecol. 54: 219-223.
- Round, F.E. 1981: The ecology of the algae.- Cambridge University Press, Cambridge.
- Shannon, C.E. and Weaver, W. 1949: The mathematical theory of communication.- Univ. Illinois Press., 117 pp. Urbana.
- Simonsen, R. 1959: Neue Diatomeen aus der Ostsee I.- Kieler Meeresforsch. 15:74-83.
- Simonsen, R. 1960: Neue Diatomeen aus der Ostsee II.- Kieler Meeresforsch. 16:126-130.
- Simonsen, R. 1962: Untersuchungen zur Systematik und Ökologie der Bodendiatomeen der westlichen Ostsee.- Int. Rev. ges. Hydrobiol., Syst. Beihefte I. 143 pp.
- Siver, P.A. 1977: Comparison of attached diatom communities on natural and artificial substrates.- J. Phycol. 13:402-406.
- Sullivan, M.J. 1975: Diatom communities from a Delaware salt marsh.- J. Phycol. 11:384-390.
- Sullivan, M.J. 1978: Diatom community structure; taxonomic and statistical analyses of a Mississippi salt marsh.- J. Phycol. 14:468-475.
- Sullivan, M.J. 1981: A preliminary checklist of marine benthic diatoms of Mississippi.- Gulf Res. Reports 7:13-18.
- Sullivan, M.J. 1982: Distribution of edaphic diatoms in a Mississippi salt marsh: A canonocal correlation analysis.- J. Phycol. 18:130-133.
- Sundbäck, K. 1979: Mikrobentisk primärproduktion, klorofyll a och artsamansättning i Östersjönområdet.- M.Sc. thesis, 64 pp. University of Turku, Finland.
- Sundbäck, K. 1982: Benthiska diatomer i Öresund - några identifikationsproblem.- In Håkansson, H. (ed.) Report 22 from Dept of Quatern. Geol., University of Lund, pp. 103-105.
- Sundbäck, K. 1983: Seasonal variation in microphytobenthic primary production and chlorophyll a content on a sandy coast with shallow brackish water, S Öresund, Sweden.- Part of doct. thesis, Univesrity of Lund, Sweden.
- Sundbäck, K. and Persson, L-E. 1981: The effect of microbenthic grazing by an amphipod, Bathyporeia pilosa, Lindström.- Kieler Meeresforsch., Sonderh. 5:573-575.
- Taasen, J.P. and Høisaeter, T. 1981: The shallow-water soft-bottom benthos in Lindåspollene, Western Norway. 4. Benthic marine diatoms, seasonal density fluctuations.- Sarsia 66:293-316.
- Thomas, D.P. 1979: Some problems connected with use of cleaned cells and relative proportions of species in diatom ecology.- Nova Hedwigia, Beiheft 64:503-511.
- Tippet, R. 1970: Artificial surfaces as a method of studying populations of benthic micro-algae in fresh water.- Br. phycol. J. 5:187-199.
- Tropper, C.B. 1975: Morphological variation of Achnanthes hauckiana (Bacillariophyceae) in the field.- J. Phycol. 11:297-302.
- Wachenfeldt, von T. 1975: Marine benthic algae and their environment in the Öresund.- Doct. thesis, 328 pp., University of Lund, Sweden.
- Weitzel, R.L. (ed.) 1979: Methods and measurements of periphyton communities: A review.- ASTM Special Technical Publication 690, 183 pp. Baltimore.
- Werff, van der A. 1960: Die Diatomeen des Dollart-Emsgebietes.- Verh. ned. geol. Mijnb. Genoot. (Geol. Ser.) 19:153-201.
- Werff, van der, A. and Huls, H. 1957-1974: Diatomeëenflora van Nederland.- Den Haag.

SPATIAL VARIATION IN CHLOROPHYLL A CONTENT
AND SPECIES COMPOSITION OF DIATOM ASSEMBLAGES
IN RELATION TO SEDIMENT CHARACTERISTICS IN A
NON-TIDAL SHALLOW BAY, S ÖRESUND, SWEDEN

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ABSTRACT

The spatial variation of two microphytobenthic variables, chlorophyll a content and species composition of diatoms, was studied in relation to sediment characteristics by sampling at 50 points within an area of 200 x 200 m in a shallow sandy bay. The following environmental variables were considered: median grain size, sorting coefficient, loss on ignition, water depth, distance from shore and sediment type (judged by eye). Chlorophyll a content varied between 23 and 258 mg m⁻² (CV=41%) within the study area and was significantly correlated with all environmental variables measured, but no single sediment variable was highly significant. The highly significant correlation between chlorophyll a content and sediment type indicates that the distribution of the microphytobenthos is affected by a combination of several factors related to degree of exposure to wave action. Ordination and classification of the species composition showed that the main type of variation was a quantitative change that formed a continuum following the exposure gradient. Exposure to wave action restricted the number of protruding life forms, colonies and motile species. There was a break in the continuum near the shore, probably connected with changed chemical conditions in the sediment, i.e. oxygen depletion and sulphide formation arising from decaying macroalgae.

1. INTRODUCTION

Heterogeneous, patchy and mosaic distribution are terms often used in describing the spatial variations in the microphytobenthos in shallow intertidal areas (for review see van den Hoek et al., 1979). Continuous gradual changes of distribution have been described along environmental gradients, for instance salinity (McIntire, 1978), tidal level and degree of exposure (Cadée and Hegeman, 1977; Colijn and Dijkema, 1981). It has also been suggested that the distribution of benthic microflora is correlated with sediment structure (Amspoker and McIntire, 1978; Colijn and Nienhuis, 1978). Two types of heterogeneous variation seem to exist, i.e. intermediate-scale and small-scale variation (van den Hoek et al., 1979). The latter, often referred to as 'micro-patchiness', implies that a great number of samples must be taken to get representative biomass measurements even within a small sampling site. On tidal flats, where it is easy to sample during low tide, a large number of samples can be collected and pooled to avoid the effect of horizontal variations in the microflora. In non-tidal areas, however, sampling is carried out by wading or diving, which makes the collecting procedure time-consuming, and rather unpleasant during cold weather. Consequently the measurements are often based on only a few replicates, which increases the effect of patchiness. Theoretically the heterogeneous distribution of the microphytobenthos may be even greater at least in sheltered non-tidal areas owing to the lack of regular tidal mixing of the substrate.

The investigation was carried out in shallow water on the northern coast of the Falsterbo peninsula, SW Sweden (Fig. 1), in the transition zone between the Baltic and Öresund (the Sound). Öresund is a shallow, virtually non-tidal strait, characterized by horizontal and vertical salinity gradients. For further details of hydrography see von Wachenfeldt (1975) and Öresundskommissionen (1980). A general description of the Falsterbo area is given by von Wachenfeldt in Jansson et al. (1982).

The aims of the investigation were:

- (a) to estimate the degree of and type of *intermediate-scale* horizontal variation of the microphytobenthic biomass (chlorophyll a) and species composition within a restricted non-tidal area;
- (b) to determine whether this variation is related to differences in the sediment characteristics;
- (c) to investigate whether the degree of *small-scale* variation is dependent on sediment type and
- (d) to examine the value and reliability of the previous investigations on the seasonal fluctuations of the microphytobenthos in the same area (Sundbäck, 1983a, b).

2. MATERIAL AND METHODS

2.1. Sampling

The sampling station is located in shallow water (Fig. 1); for hydrography and microbenthic primary production see Sundbäck (1983a). The site was chosen because (a) it is shallow enough to enable sampling over a sufficiently large area by wading, (b) the type of sediment varied and (c) a sampling station from a previous investigation (Sundbäck, 1983a, b) had been situated here (see Fig. 2).

The sampling for *intermediate-scale* variation was done on June 30, 1982 and samples for *small-scale* horizontal distribution and vertical distribution were taken on August 18, 1982. Five transects 40 m apart were sampled at 20 m intervals (a measuring tape was used), i.e. 50 points within a 200 x 200 m area (Fig. 2).

Table 1 lists the variables estimated with units and abbreviations. Samples for chlorophyll a and species composition analyses were taken with a piston corer (a cut-off 2 ml syringe, ID 9 mm) from the topmost 5 mm of sediment: three samples for chlorophyll a and three samples for species composition at each point. At every second sampling point a sediment core (70 mm ID) c. 40 mm deep was taken for analysing grain-size distribution and loss on ignition. The depth of water at each

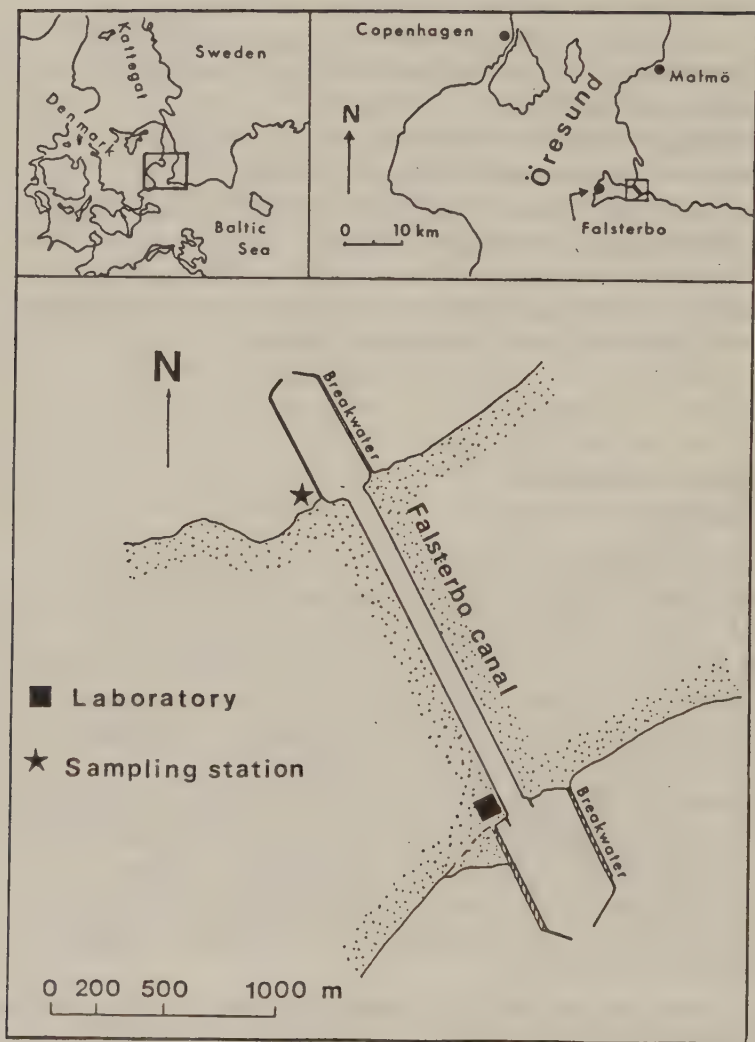


Fig. 1. Location of the sampling station on the Falsterbo peninsula, SW coast of Sweden.

Table 1. Variables estimated (abbreviations in brackets) and species composition analyses used. Only the two first variables included in the August sampling.

Variable		Unit	Number of points sampled
Chlorophyll <u>a</u>	(Chl)	mg m ⁻²	50
Pheopigment	(Pheo)	mg m ⁻²	50
Loss on ignition	(Ign)	%	25
Median grain size	(MedGr)	mm	25
Sorting coefficient	(Sort)	σ_g	25
Water depth	(WD)	m	50
Distance from shore	(Dist)	m	50
Sediment type	(SedType)		50
Analyses for species composition:			
Relative abundance	(RA, %)		37
Diversity	(H')		37
Evenness	(J')		37
Classification, TABORD			37
Ordination, ORDINA			37

sampling point was measured to obtain a relative measure of the slope and notes on the sediment type were made subjectively, (i.e. colour, softness) and macroflora. A layer of loose filamentous brown algae was drifting in the bay on the sampling occasions, shading at least occasionally parts of the sampling area.

With the help of notes made in the field a map of the sampling area was constructed to show the four types of sediment that were distinguished subjectively (Fig. 2):

- I clean pale sand, mostly without benthic macroflora, farthest out;
- II slightly darker sand, intermediate between types I and III, with patches of macroflora, in the middle of the study area;
- III softer, darker sand with patches of *Ruppia* sp., near the shore;
- IV dark sand with patches of *Ruppia* near the shore and along the breakwater.

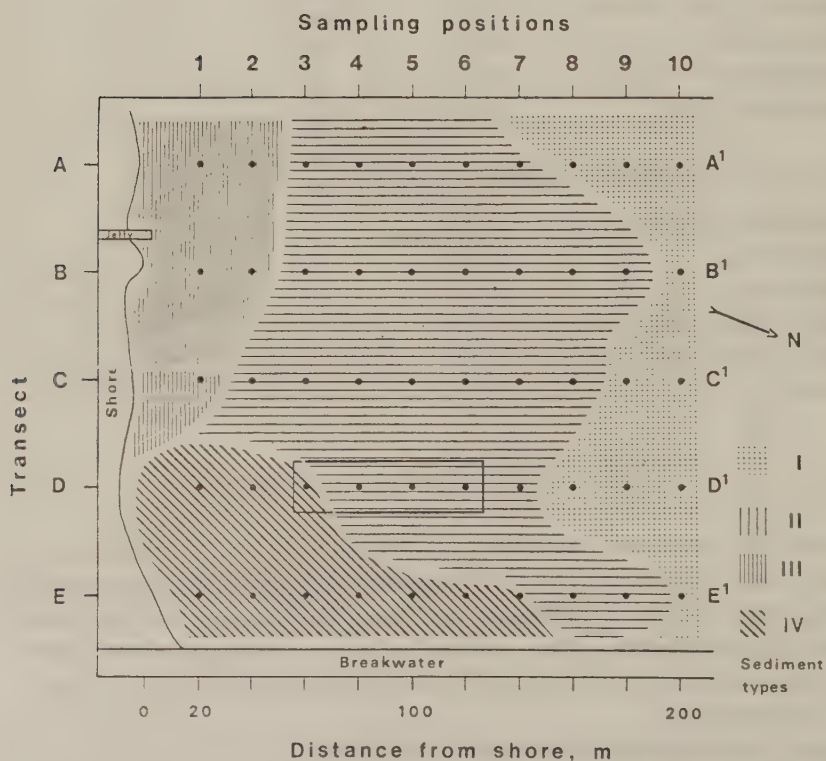


Fig. 2. Sampling positions along five transects, and the four sediment types (see 2.1.). The rectangle denotes the approximate position of station 2 in Sundbäck (1983a,b).

The small-scale variation in chlorophyll a content in the top 5 mm of sediment was studied within three areas of 0.25 m², each marked out by means of a 50 x 50 cm metal frame divided by wires into 100 5 x 5 cm squares, on three subjectively chosen sediment types: type I (position C9 in Fig. 2), type III (position B2) and type IV (position D2). Three 0.64 cm² samples were taken within 10 such 5 x 5 cm squares randomly selected with the aid of a calculator, and the samples were pooled.

Samples for investigating vertical distribution in the top 3 cm of sediment were taken with the piston corer on the three sediment types and 1 cm at a time pressed out, except for the top cm which was pressed out in two 5 mm lots. Again three samples were pooled for each section. To get chlorophyll a samples from deeper layers, two cores, one in type I, the other in type III, were taken with a PVC tube (60 mm ID) lined with a plastic bag.

All samples were transported to the laboratory in thermally isolated boxes and stored cool and dark until the next day. The sediment cores for vertical chlorophyll a distribution were frozen for later handling. Before analyses they were slightly thawed, so that the plastic bag containing the sediment could be pulled out. The sediment was cut into 1 cm slices. Only the centre of each slice was used in the analyses to avoid contamination. The top two cm were not used because the surface of the cores was disturbed.

2.2. Analyses

Chlorophyll a and pheopigment contents were analysed according to Lorenzen (1967) (see Sundbäck, 1983a). Loss on ignition was measured as percentage of weight loss of dry sediment (105°C) after 2 hours at 550°C.

Grain size distribution was determined by sieving dry sediment through screens with 0.4, 0.25, 0.2, 0.15 and 0.1 mm mesh. The fractions were expressed as percentage of the total dry weight of the sample. Median grain size and sorting coefficient (graphic standard deviation) were determined from cumulative curves (see Gray, 1981).

The BMB programs P6D, P2R and P3S (Dixon, 1981) were used to calculate correlations between variables.

2.3. Species composition

The species composition of the benthic microflora was studied in four steps:

(1) Fresh sediment samples were examined under the microscope ($\times 125$ and $\times 500$ magnification) to obtain a rough estimation of the proportions of empty diatom frustules and organisms other than diatoms.

(2) Subsamples of homogenized sediment samples were oxidized (H_2O_2) and permanent slides prepared for diatom counts (for details see Sundbäck, 1983b). Additional slides were prepared by incineration at 550°C to examine colonies of diatoms. The relative abundance (RA, %) was based on 350-400 valves per slide. A general idea of the variation in species composition was investigated in sediment from the 25 sampling points where sediment structure had been analysed. Since this preliminary analysis showed that there was a pattern of distribution, an additional 12 sampling points, chosen subjectively from transition zones between sediment types, were analysed for the final numerical computations (see below).

(3) Although the species composition material came from only 37 sampling points and comprised only 74 taxonomic units (after exclusion of rare taxa; see below), the distributional pattern was not obvious from a study of the species lists. Therefore numerical computer-assisted analyses were used, i.e. classification, ordination and diversity calculations. For classification the program TABORD (Persson, 1977; van der Maarel et al., 1978), which produces a phytosociological table, was used. The ordination program ORDINA (Roskam, 1971) produces ordination diagrams which were here used to study distributional trends (for further information see Persson, 1980 and Johansson, 1982). Computations of classification and ordination were run according to the manuals by Persson (1977, 1978) at the Lund University Computer Centre. The Shannon diversity index H' and the evenness index J' (equations from Liljelund, 1977) were chosen to describe diversity. For principles of data editing and computing see Sundbäck (1983b).

(4) The mean relative abundance of the constant species of each cluster created by the classification was recalculated taking cell volume into consideration. This was done to roughly esti-

mate the proportion of the different species (with different cell volumes) in the total biomass of the diatoms. Cell volumes were calculated according to recommendations in Edler (1979) (for details see Sundbäck, 1983b).

2.4. Sediment characteristics of the study area

Grain size distribution changed gradually from the shore seawards (Fig. 3). Median grain size for the whole investigation area varied between 0.18 and 0.29 mm, i.e. between fine and medium sand, being smallest in type III and type I sediment. The largest median grain sizes were found in dark sand near the shore along transects D-D' and E-E'.

Sorting coefficients ranged from 0.23 to 0.61 and indicated well sorted sediment (0.35-0.50) at most sampling points.

Very well sorted sediment (<0.35) was found on type I and type II and moderately well sorted (>0.50) on type III and IV (for sorting classes see Gray, 1981).

Loss on ignition was low, varying between 0.28% of dry weight on type I and 1.07% on type IV.

On the basis of sediment characteristics the study area can be described as a sheltered bay with fine sand. On the sampling occasions there were no ripple marks on the landward side of a sandbank. The median grain size is often close to 0.18 mm (the particle size easiest to move; Gray, 1981), thus indicating stable conditions. On the other hand the sediment is well sorted, which is considered characteristic of high wave and current velocity, indicating that sorting occurs during winter gales, for instance.

The macrovegetation consisted of *Ruppia* sp. but *Chara* sp. also occurred sparsely on all sediment types. *Fucus vesiculosus* was found growing attached to mussels buried in type II sand along transects A-A' and B-B'. The filamentous brown algae *Pilayella* sp. and *Ectocarpus* sp. were either epiphytic on *Fucus* or drifting, covering about half of the area sampled. Patches of purple bacteria occurred on the dark sand near the shore (type IV) and also along the inner edge of a sandbank c. 180 m from the shore at C9 (Fig. 2). In late summer loose

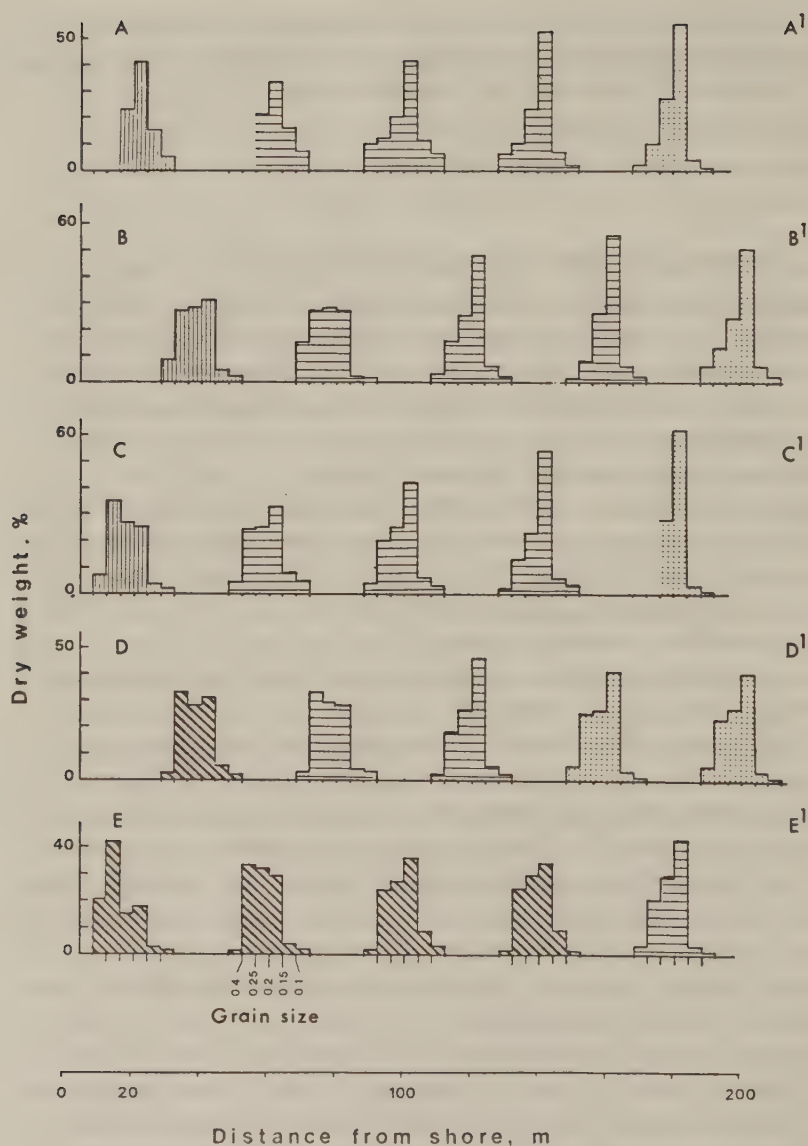


Fig. 3. Grain size distribution at 25 sampling points along five transects (A-A', etc.). Unfinished outlines in A-A' and C-C' indicate that the 0.4 mm sieve was not used. Different types of shading represent different sediment types (see Fig. 2).

decaying macroalgae usually begin to accumulate in the inner part of the bay (transects D-D' and E-E'). On the sampling occasions no large amounts of macroalgae had yet accumulated but purple bacteria and the smell of H_2S indicated anoxic conditions. Depth of water on the sampling occasion in June varied from 0.05 m to 0.65 m between the sampling points, the depth gradually increasing seawards.

3. RESULTS

3.1. Horizontal distribution of chlorophyll a

Chlorophyll a values ranged from $23 \text{ mg} \cdot \text{m}^{-2}$ ($3 \text{ } \mu\text{g} \cdot \text{g}^{-1}$) on type I along transect C-C' to $258 \text{ mg} \cdot \text{m}^{-2}$ ($33.5 \text{ } \mu\text{g} \cdot \text{g}^{-1}$) on type III along transect E-E' (Fig. 4). The mean value for the whole area was $90.5 \text{ mg} \cdot \text{m}^{-2}$ ($11.8 \text{ } \mu\text{g} \cdot \text{g}^{-1}$) (CV=41.2%). Variation was slight along transect A-A' and greatest along transect E-E'. Extreme chlorophyll a values, as at E2 (Fig. 4), may occur as a result of the accumulation of dead microphyto-benthic cells that still contain measurable chlorophyll a (see van den Hoek et al., 1979), and the high values could be an artifact due to cells having been killed by oxygen depletion as indicated by the presence of purple sulphur bacteria and the smell of H_2S . However, microscopic examination of fresh samples revealed many viable diatom cells (e.g. small motile cells of *Nitzschia*) caught up in detritus floccules between the sand grains. These densely 'inhabited' floccules were obviously responsible for the high chlorophyll values. The spectrometer readings at 750 nm (correction for turbidity) were markedly higher on the dark sand indicating interference by bacteriochlorophyll from purple bacteria, which has its maximum absorbance at 770 nm in extraction (Fenchel and Straarup, 1971).

As with sediment characteristics, chlorophyll a values of samples from points D_3 - D_6 (identical with station 2 in Sundbäck 1983a), agree well with those obtained at the same time of the year at station 2.

Pheopigment values varied between 0 and $44 \text{ mg} \cdot \text{m}^{-2}$ ($\bar{x}=16.7$, CV=61%), with a tendency for values to decrease seawards (Fig. 4). Near the shore on transects D-D' and E-E', however, no pheopigment was detected in the sediment.

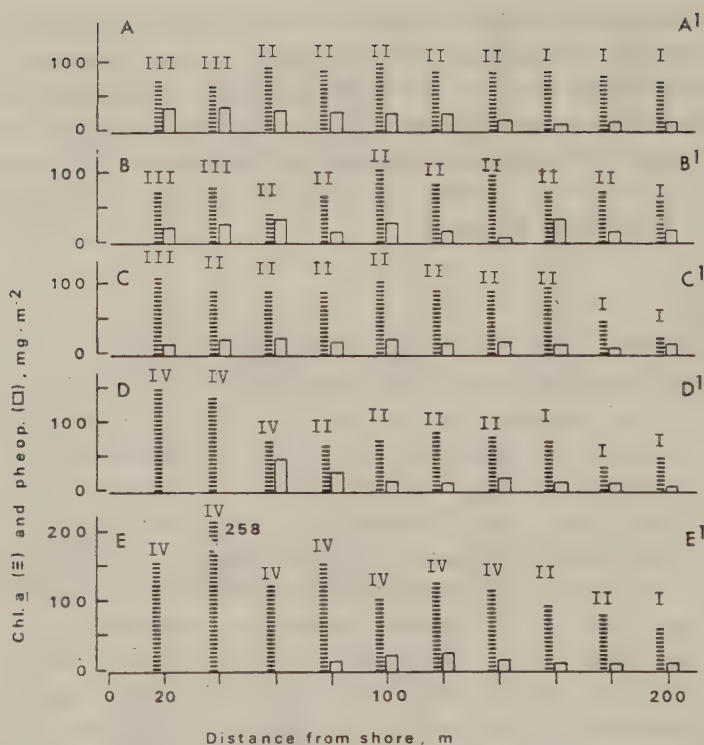


Fig. 4. Chlorophyll a and pheopigment contents at 50 points along 5 transects. Roman numerals indicate sediment type (see Fig. 2).

3.2. Small-scale horizontal variation in chlorophyll a content

The samples for small-scale variation in chlorophyll a content were taken 7 weeks later than the transect samples. Again, the chlorophyll a content was highest in the dark sediment near the shore (type IV). The degree of variation was the same (CV=12-13%) in all three sediment types (Table 2). Samples taken close together did not differ less than samples from opposite sides of the square. The pheopigment content was higher and varied less in type III sediment than in type I and type IV, where low values were found (Table 2).

Table 2. Small-scale variation in chlorophyll a and pheopigment content. Three samples were pooled in each of ten points randomly chosen within a 0.25 m² square in each of three sediment types. CV = coefficient of variation.

Sediment type	Chl <u>a</u> , mg m ⁻²				Pheop., mg m ⁻²		
	<u>n</u>	<u>$\bar{x}$</u>	Range	CV, %	<u>$\bar{x}$</u>	Range	CV, %
Type I	10	80.5	66.7- 94.4	11.8	1.9	0- 5.9	128.6
Type III	10	48.3	36.8- 58.0	13.2	17.8	12.6-25.8	21.4
Type IV	10	120.9	102.2-148.0	13.0	4.8	0-29.4	190.3

3.3. Vertical distribution of chlorophyll a

The vertical profile of chlorophyll a was similar in type I and type III sediment (type IV was sampled only down to 3 cm). The top 2 cm contained about 50% of the total amount of chlorophyll a down to 10 cm (Fig. 5). About 70% of the chlorophyll a in the top 1 cm was found in the uppermost 0.5 cm which corresponds to the depth of the samples taken in my earlier investigations. Below 5 cm sediment depth the chlorophyll a content decreased markedly in soft sediment, while in type I the amount remained the same down to 17 cm. Highest pheopigment values were found a few centimetres below the sediment surface (Fig. 5). The pheopigment profile was steeper in type III sand than in type I sand. The high value below 15 cm in clean sand was due to buried fragments of macroalgae. Sediment from depths of more than 10 cm was examined under the microscope for apparently living microalgae, i.e. with intact chloroplasts. In sediment type I living diatom cells (mainly *Fragilaria* spp.) were still found in the 16-17 cm layer, i.e. the deepest layer samples. In type III, near-shore sediment, fewer living cells were found in the deepest sections (down to 13 cm).

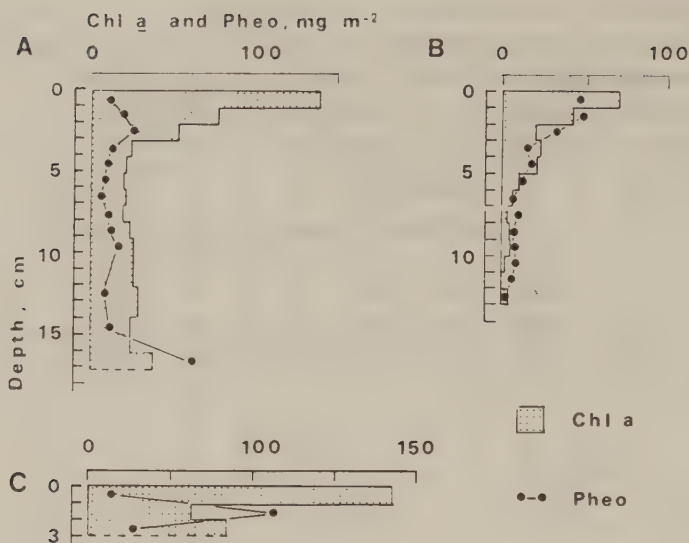


Fig. 5. Vertical distribution of chlorophyll *a* (Chl) and pheopigment (Pheo) in three sediment types: A = type I, B = type III and C = type IV (see 2.1.).

3.4. Characteristics of the microflora based on fresh samples

The proportion of suspendible fraction (Cadée and Hegeman, 1974) increased gradually from the sandbank to the shore. On sediment type I the sand was seen to be clean under the microscope. Flat diatoms attached to the cavities of the sand grains dominated the microflora. The amount of suspendible matter was slight. On type II there was more suspendible matter, e.g. detritus floccules and empty diatom frustules. Cyanophytes (Cyanobacteria), e.g. *Microcystis* sp., *Merismopedia* sp., *Anabaena* sp. and *Oscillatoria* sp. were attached to sand grains. In addition to flat diatoms the well-developed epipsammic flora included single cells and colonies of rapheless genera (*Fragilaria* and *Opephora*) protruding from the surface of the sand grains. On type III the sand grains had a woolly appearance

due to the presence of bacteria, fungi and unidentifiable matter. *Fragilaria*-like colonies dominated the epipsammic flora. Large naviculoids were more frequent than in types I and II. The suspendible fraction comprised detritus floccules and diatom frustules, roughly 50% of which were empty. Cyanobacteria occurred as in mixed sand.

On type IV in spite of the smell of H_2S and the presence of purple bacteria there was a rich microflora. The sand grains had large colonies of purple bacteria and diatoms (*Fragilaria* and *Opephora*). In the suspendible fraction the detritus floccules were densely inhabited by diatoms, both attached and motile. Small motile *Nitzschia*-like species dominated the motile species. Cyanophytes were also frequent.

3.5. Numerical analysis of the diatom flora

The phytosociological table produced by the TABORD program (Table 3) gives a general view of the species composition. All quantitative analyses are based on relative abundance calculated from cell counts. Varying cell sizes of the different taxa were not taken into account at this stage. The 74 taxonomical units included in the analyses are listed in the Appendix.

3.5.1. Classification

The fusion of the samples into groups by the classification program was stopped when 4 clusters remained, which happened at a similarity ratio (range 0-1) of 0.878 (Table 4). The species composition consisted mainly of c. 20 constant species (=species with at least 70% frequency in a cluster) making up c. 90% of the total mean cell count in each cluster. The main differences between the clusters are due to different proportions of these species (Table 3). If we define a dominant species as one with a relative abundance of $\geq 10\%$ at least once in the cluster there are two such species present in all four clusters: *Fragilaria pinnata* and *Opephora martyi* (cf. the *F. pinnata* - *O. martyi*-association, Sundbäck, 1983b). The clusters are, however, differentiated out on

Table 3. Classification of 37 sampling points by the TABORD program. Digits represent relative abundance classes (- to A): (-) RA <1% and (A) RA >25%. Species with a mean relative abundance of $\geq 10\%$ in a cluster are underlined. Only part of the TABORD table is shown.

Species	Cluster			
	111111111111111	222222	333333333333	444
<i>Amphora coefferaeformis</i>	- - - - -	- - -	- - - - - 1 -	- 1
<i>Amphora tenerima</i>	1-2-1--11--11-1	- -	111--11--	212
<i>Navicula hansenii</i>	2-- - - - -	- - -	- - -	-
<i>Achnanthes hauckiana</i>	<u>566578875885777</u>	<u>8767677</u>	655437725477	324
<i>Achnanthes lemmermannii</i>	<u>76667677778676</u>	4554555	5654454333566	-12
<i>Achnanthes cf. tenera</i>	22222422211242	22--21	443-23311333	2-2
<i>Amphora coff. var. perpusilla</i>	- -11- 21--1--1	--11-	- 11--12--	123
<i>Amphora exigua</i>	11222132-111-21	11-2111	-1--1--1	211
<i>Amphora sp. 1</i>	226252233211512	-212-22	23432152-2-	<u>855</u>
<i>Cocconeis sp. 1</i>	- - - - - 1-1-	1--1-11	- - - - -	-
<i>Cocconeis placentula et var.</i>	- - - - - 1-111--	1-111--	-3-- - - - 1--	-
<i>Fragilaria pinnata</i>	355556336677555	<u>7898798</u>	<u>AA9AA99AA8A</u>	<u>895</u>
<i>Navicula amphipleuroides</i>	- - - - - 1-1-- 1 --	--2111	- - -21--	212
<i>Navicula cf. biskanteri</i>	464233443423253	5332212	434343422-41	422
<i>Navicula crucifera</i>	- - - - -	- - - - -	- - - - -	-
<i>Navicula cryptocephala et var.</i>	2212-11132221-2	121111	12-11--1121	1-2
<i>Navicula cryptolyra</i>	1--1-1 -- 1121	- - - - -	- -1112212-	-13
<i>Navicula florineae</i>	1--1-- - - -	- - - - -	- - - - -	-
<i>Navicula phyllepta</i>	1-- - - - - 1--	- - - - -	- 1-- - - - 1--	-
<i>Navicula salinarum et var.</i>	443563662423624	3434444	12321231-331	211
<i>Navicula sp. 5</i>	2-2425334343355	-122222	-2-321333322	113
<i>Navicula subinflata</i>	- - - - -	- - - - -	- - - - -	-
<i>Navicula sp. 1</i>	1111224-1222142	222-122	222122---21	--
<i>Nitzschia frustulum var. subsalina</i>	554562435664637	<u>8786667</u>	4351511- 1-5	--
<i>Nitzschia pusilla</i>	454333254344434	3344533	435663576443	<u>8A8</u>
<i>Opephora martyi (incl. olseni-type)</i>	655535333245433	5557847	533537777655	467
<i>Diploneis didyma</i>	-	- - - - -	- - - - -	-
<i>Mastogloia exigua</i>	- - - - -	- - - - -	- - - - -	-
<i>Navicula arenicola</i>	- - - - -	- - - - -	- - - - -	--1
<i>Navicula cruciloides</i>	- - - - -	- - - - -	- - - - -	-
<i>Opephora schulzii</i>	- - - - -	- - - - -	- - - - -	-
<i>Synedra tabulata et var.</i>	- - - - -	- - - - -	- - - - -	-
<i>Achnanthes pseudosolea</i>	- - - - -	- - - - -	- - - - - 1--	-
<i>Fragilaria virescens var. subsalina</i>	- - - - -	- - - - -	- - - - - 1-2--	111
<i>Amphora abludens</i>	- - - - -	- - -	- - - 1 -	--1
<i>Fragilaria construens var. venter</i>	- - - - -	- - -	- - - - -	1--
<i>Cocconeis stauroneiformis</i>	43- - -	- - -	- - -	-

Table 4. Similarity ratios (SR, range 0-1) between the four final clusters produced by the TABORD program.

Cluster	1	2	3	4
1	1.000			
2	0.845	1.000		
3	0.635	0.800	1.000	
4	0.462	0.510	0.650	1.000

relative abundance of the other common species. When cell volume is considered the picture remains essentially the same except that the importance of *F. pinnata* is reduced and some larger species, e.g. *Amphora exigua* *F. virescens* var. *subsalina* make up a larger proportion (Table 5).

The constant species, which were also most abundant, were small with a cell length of less than 20 μm and a cell volume between c. 50 and 100 (200) μm^3 . *Fragilaria pinnata*, one of the dominant species, occurred as almost round cells only a few μm long with cell volume of c. 20 μm^3 .

The constant species were mainly represented by three life forms (see Sundbäck, 1983b):

(a) Flat cells attached to the cavities of the sand grains, (*Achnanthes hauckiana*, *A. lemmermannii*, *Navicula* cf. *biskanteri* and *Navicula* sp. 5).

(b) Rapheless attached species (*Fragilaria* spp. and *Opephora* spp.), usually forming raised colonies on the sand grains.

(c) Small motile life forms, here represented by two *Nitzschia* species.

(d) Large motile species and (e) epipsammic species were less important constituents of the microphytobenthos. The proportions of the three main life forms changed gradually from cluster 1 to cluster 4 (Fig. 6). The gradual change in the proportions of the diatom species and life forms agree well with the observations on fresh samples. However, in the more sheltered near-shore habitat, the proportions of protruding cells (life form (b)) and motile forms (life form (c)) may

Table 5. Proportions of the commonest species expressed as relative abundance based on cell counts and cell volume. For cell volume only constant species are included (c. 90% of the total cell count).

	Relative abundance based on cell counts, %		Relative abundance based on cell volume, %
Cluster 1	<i>Achnanthes lemmermannii</i> 11.2	<i>Achnanthes hauckiana</i>	12.6
	<i>Achnanthes hauckiana</i> 11.0	<i>Achnanthes lemmermannii</i>	11.2
	<i>Fragilaria pinnata</i> 8.6	<i>Opephora martyi</i>	10.3
	<i>Nitzschia frustulum</i>	<i>Nitzschia pusilla</i>	7.3
	var. <i>subsalina</i> 7.4	<i>Amphora exigua</i>	6.5
	<i>Nitzschia pusilla</i> 6.5	<i>Navicula salinarum</i>	6.1
	<i>Opephora martyi</i> 5.8		
Cluster 2	<i>Fragilaria pinnata</i> 15.1	<i>Opephora martyi</i>	15.8
	<i>Achnanthes hauckiana</i> 11.8	<i>Achnanthes hauckiana</i>	12.6
	<i>Nitzschia frustulum</i>	<i>Nitzschia frustulum</i>	
	var. <i>subsalina</i> 11.8	var. <i>subsalina</i>	
	<i>Opephora martyi</i> 9.4	<i>Achnanthes lemmermannii</i>	7.7
	<i>Achnanthes lemmermannii</i> 8.1	<i>Nitzschia pusilla</i>	6.0
Cluster 3	<i>Fragilaria pinnata</i> 22.6	<i>Opephora martyi</i>	17.2
	<i>Opephora martyi</i> 8.6	<i>Achnanthes hauckiana</i>	10.6
	<i>Achnanthes hauckiana</i> 8.4	<i>Nitzschia pusilla</i>	10.4
	<i>Nitzschia pusilla</i> 8.3	<i>Achnanthes lemmermannii</i>	8.2
	<i>Achnanthes lemmermannii</i> 7.4	<i>Fragilaria pinnata</i>	7.0
	<i>Navicula biskanteri</i> 4.9	<i>Fragilaria virescens</i>	
		var. <i>subsalina</i>	5.1
Cluster 4	<i>Nitzschia pusilla</i> 18.0	<i>Nitzschia pusilla</i>	19.6
	<i>Fragilaria pinnata</i> 13.0	<i>Opephora martyi</i>	16.4
	<i>Amphora</i> sp. 1 10.1	<i>Fragilaria virescens</i>	
	<i>Opephora martyi</i> 9.4	var. <i>subsalina</i>	10.8
	<i>Achnanthes hauckiana</i> 4.9	<i>Amphora</i> sp. 1	8.2
		<i>Achnanthes hauckiana</i>	5.4

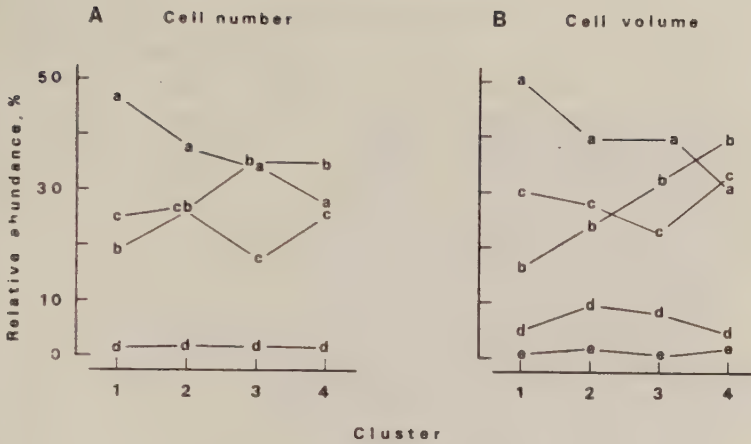


Fig. 6. Change in proportions of life forms of benthic diatoms from cluster 1 to cluster 4 based on cell counts (A) and cell volume (B). a = flat attached species (epipsammic), b = small rapheless attached species forming raised colonies on sand grains (epipsammic), c = small motile species, d = large motile species and e = large attached species.

have been overestimated owing to deposited dead cells.

Cluster 1 is related to type I and type II sediment, cluster 2 to type II but closer to the shore. Cluster 3 is related to type III and type IV and the smallest cluster (4) in sediment type IV along transects D-D' and E-E' (Fig. 7).

3.5.2. Ordination

The quantitative (relative abundance) ordination was definitely more informative than the qualitative (presence/absence) ordination. The first 4 dimensions extracted 84% of the variation of the relative abundance data but only 37% of the presence/absence data. This was not unexpected since the species composition of the diatom flora was the same for the

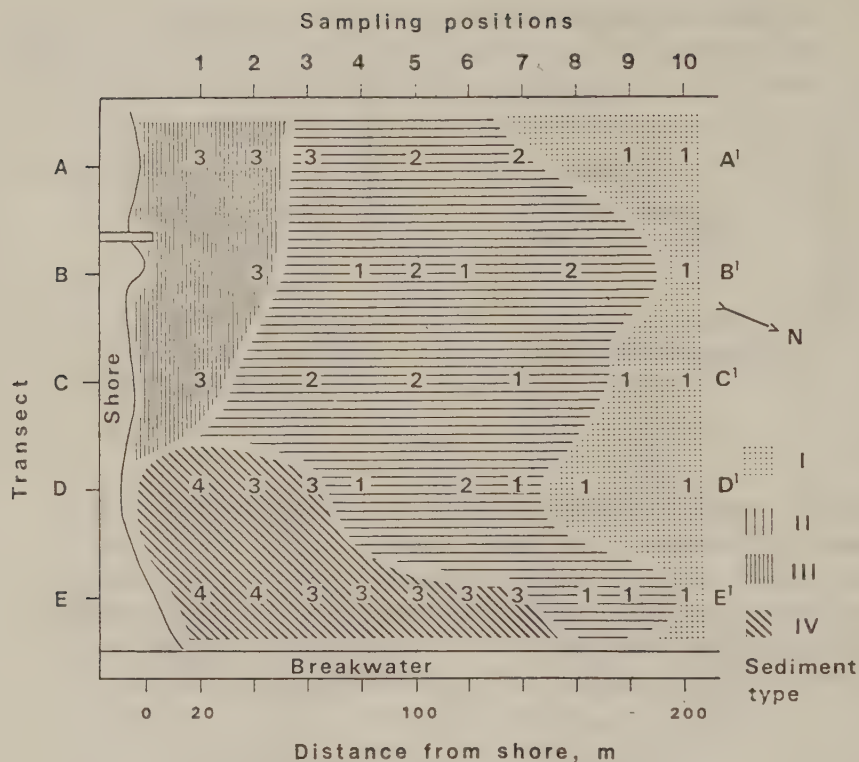


Fig. 7. Origin of clusters.

whole area. In the ordination diagram, which shows the first two dimensions (Fig. 8), the four clusters created by the classification are grouped in a horseshoe-shaped formation (typical of the type of principal component analysis used here; cf. Persson, 1977). Clusters 1, 2 and 3 form a continuum, while there is a gap between cluster 4 and the other clusters.

The first dimension accounts for about half (47.1%) of the extracted variance and presumably reflects the gradual change from exposed conditions (cluster 1 to the left in the ordination diagram) to sheltered near-shore conditions. Dimension 2 extracts 22% of the variation, but is a function of the first

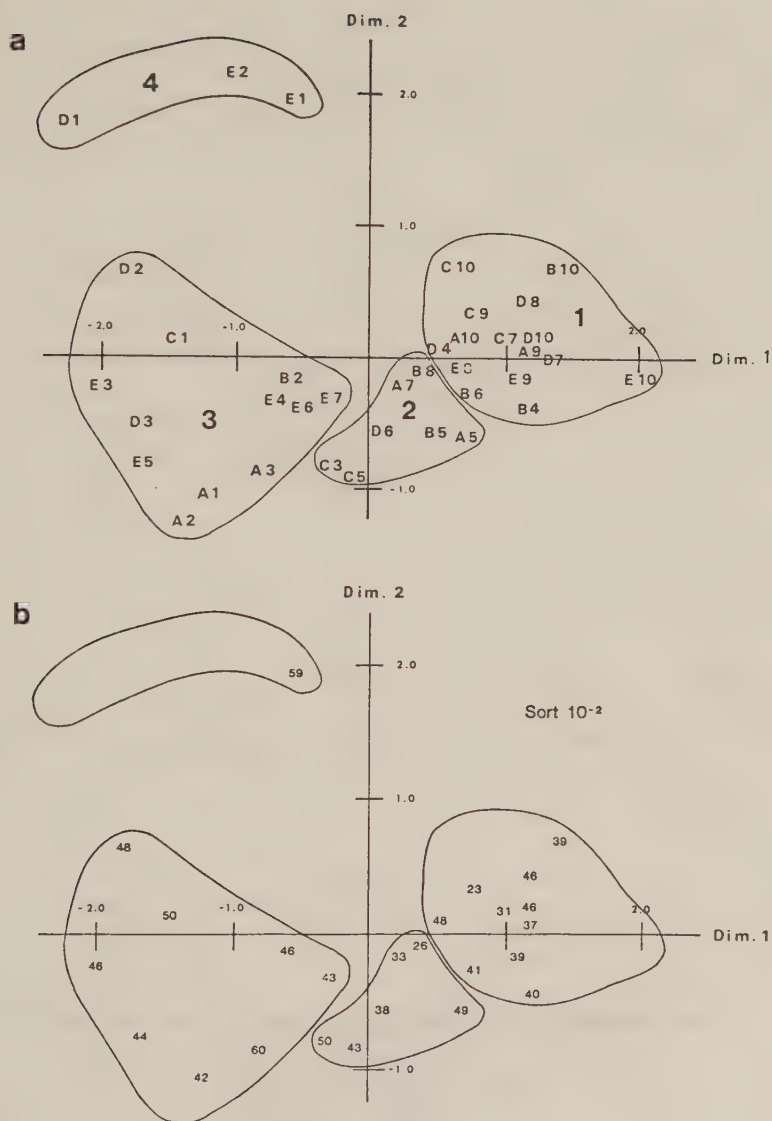
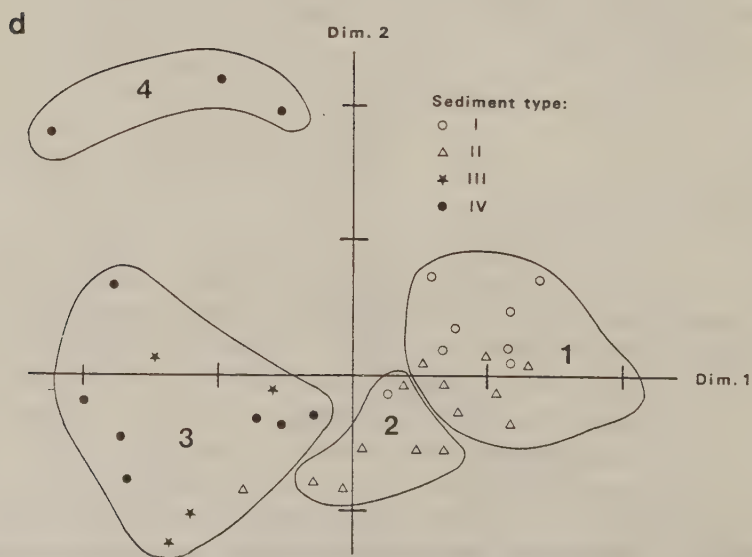
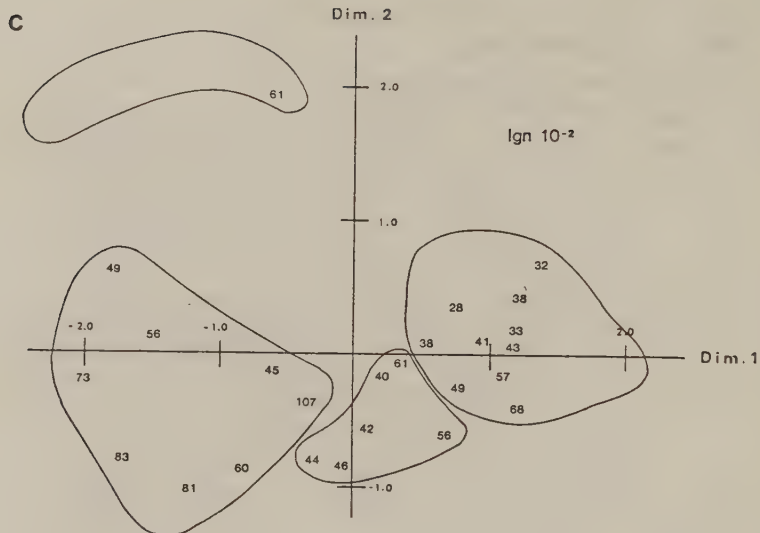
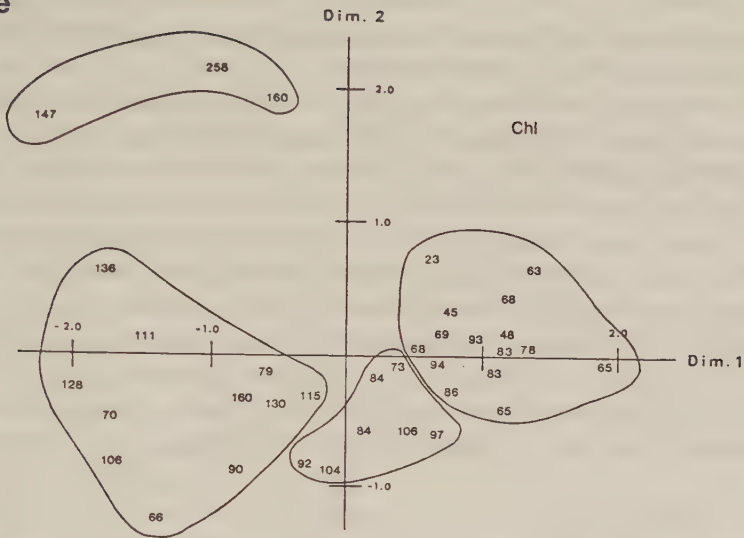


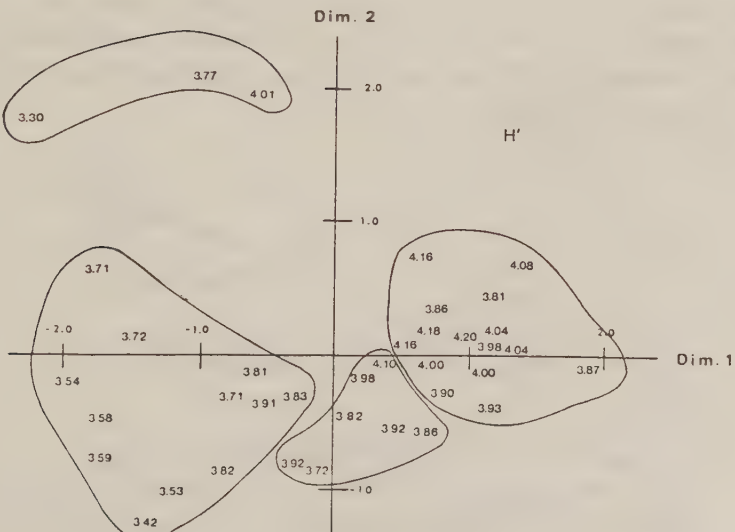
Fig. 8. Ordination (dimensions 1 and 2) of diatom samples based on relative abundance data from 37 sampling points: (a) sample numbers (D1, etc) and cluster numbers (1-4); (b) sorting coefficient; (c) loss on ignition; (d) sediment type; (e) chlorophyll a content; (f) diversity.



e



f



axis (horseshoe formation). Dimensions 3 and 4 (not shown) extract 8.4% and 6.5% of the variance respectively. This variance is not, however, interpretable.

An attempt to interpret the ordination can be made by replacing the sample numbers (Fig. 8a) by estimated values for environmental variables (Figs 8b and c). However, no single variable fits completely into the ordination, but the correlation between the subjective sediment type and the location of samples in the ordination diagram was strong (Fig. 8d). Chlorophyll *a* content in the ordination shows a clear trend, increasing from cluster 1 to cluster 4 (Fig. 8e). It can thus be seen that the trend shown by the ordination is the result of a complex of gradients related primarily to degree of exposure than to any single sediment variable.

3.5.3. Diversity

The diversity index H' varied between 3.30 and 4.20 for individual samples and tended to increase seawards. This trend is also seen in Fig. 8f, where sample numbers are replaced by H' values. The evenness index J' followed the same trend and varied between 0.66 and 0.81. Fig. 9 illustrates the differences in proportions of species abundance as mean values for each of the four clusters.

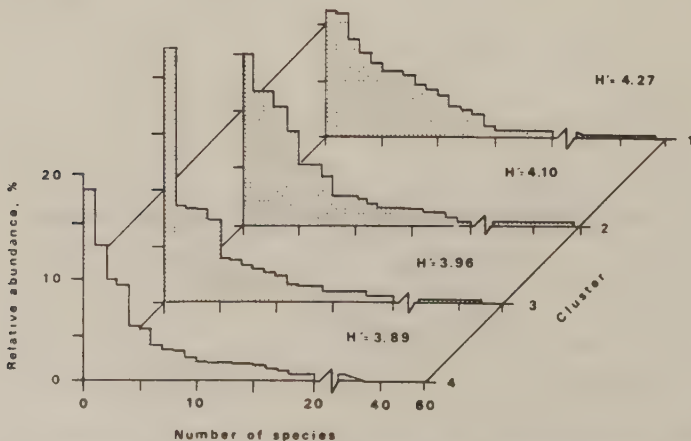


Fig. 9. Diversity of clusters shown as distribution of mean relative abundance of the species in the clusters. H' values for each cluster were calculated from mean relative abundance.

3.6. Correlation between variables

Simple correlations were calculated between all variables measured. Nonparametric and parametric tests gave essentially the same results and only one type (parametric) is shown (Table 6).

Chlorophyll a was significantly correlated with all environmental variables and also with the structural variables cluster and diversity index H' (Table 6, Fig. 10A). Of environmental variables the strongest positive correlation was obtained for the subjective variable sediment type (note that coefficients in Table 6 are based on different numbers of observations and higher significance may occur for lower values of r). Highly significant negative correlation was found for distance from shore and consequently also for depth of water (Fig. 10A). Pheopigment was significantly correlated only with median grain size and chlorophyll a content. The weak correlations may partly be due to the near-shore O-values along transects D-D' and E-E'. The bivariate plots (Fig. 11 a-d) show that the scatter around the regression lines lowers the significance of the correlations for the single sediment characteristics.

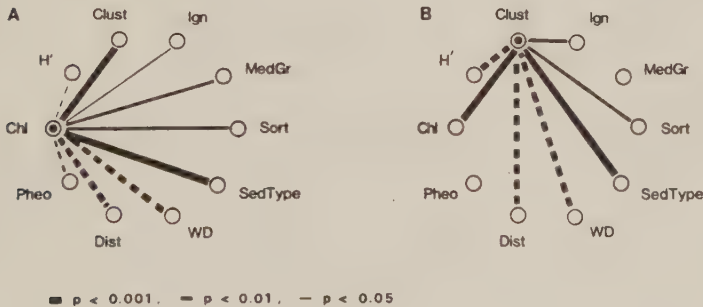


Fig. 10. Simple correlations between (A) chlorophyll a content and other variables and (B) cluster and other variables. Broken lines indicate negative correlations.

Table 6. Correlation coefficients (simple regression) between chlorophyll a content, species composition indices and environmental variables. For abbreviations of variables see Table 1. Levels of significance: a = $p < 0.001$, b = $p < 0.01$, c = $p < 0.05$, NS = nonsignificant. n = 25 for correlations including Ign, MedGr, Sort; n = 37 for correlations including Clust and H'; n = 50 for correlations including all others.

	Chl	Pheo	H'	Clust	Ign	MedGr	Sort	SedType	WD	Dist
Chl										
Pheo	-0.380 ^b									
H'	-0.332 ^c	0.060 ^{NS}								
Clust	0.704 ^a	0.001 ^{NS}	-0.683 ^a							
Ign	0.525 ^b	0.142 ^{NS}	-0.480 ^b	0.571 ^b						
MedGr	0.562 ^b	-0.505 ^c	0.026 ^{NS}	0.408 ^{NS}	0.122 ^{NS}					
Sort	0.515 ^b	-0.037 ^{NS}	-0.266 ^{NS}	0.534 ^b	0.185 ^{NS}	0.574 ^b				
SedType	0.538 ^a	-0.015 ^{NS}	-0.416 ^c	0.854 ^a	0.738 ^a	0.471 ^c	0.430 ^c			
WD	-0.623 ^a	0.171 ^{NS}	0.505 ^b	-0.822 ^a	-0.382 ^{NS}	-0.541 ^b	-0.531 ^b	-0.419 ^b		
Dist	-0.456 ^a	-0.089 ^{NS}	0.649 ^a	-0.801 ^a	-0.346 ^{NS}	-0.486 ^c	-0.627 ^a	0.488 ^a	0.783 ^a	

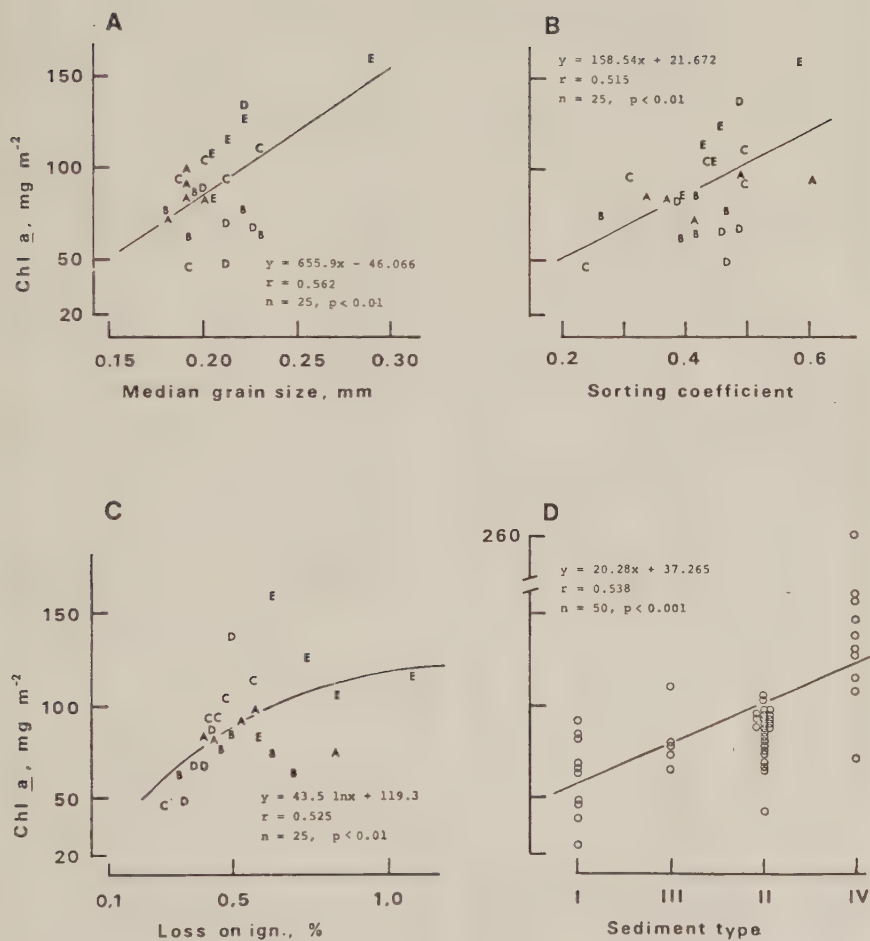


Fig. 11. Chlorophyll a content plotted against sediment characteristics: A, median grain size: B, sorting coefficient: C, loss on ignition and D, sediment type.

Table 7. Stepwise multiple regression analysis of chlorophyll a content (dependent variable) and other variables; n = 25.

A. All variables included				Partial correlation of variables not entered into equation (x 1000							
Step	Entered variable	r	Explained variance, %	Ign	MedGr	Sort	WD	Dist	SedType	H'	Clust
0				525 ^b	562 ^b	.515 ^b	-751 ^a	-580 ^b	789 ^a	NS	743 ^a
1	SedType	0.789	62.2	NS	NS	NS	-459 ^c	NS		NS	NS
2	WD	0.838	8.0	NS	NS	NS		NS		NS	NS

B. Variables Ign, MedGr and Sort included				Partial correlation of variables not entered into equation (x 1000							
Step	Entered variable	r	Explained variance	Ign	MedGr	Sort					
0				525 ^b	562 ^b	.515 ^b					
1	MedGr	0.562	31.6	475 ^c		NS					
2	Ign	0.686	15.6			NS					

Level of significance: a = p < 0.001, b = p < 0.01, c = p < 0.05, NS = not significant
Minimum F to enter value = 4.00

According to a stepwise multiple regression analysis (chlorophyll a = dependent variable) sediment type accounted for 62% of the variation and depth of water added 8% (Table 7a). If we only include the three measured sediment variables (MedGr, Sort, Ign) as independent variables, median grain size and loss on ignition accounts for 31.6% and 15.6% respectively (Table 7b). However, the multiple correlations are based on only 25 sampling points (=n), which lowers the significance of the correlation.

A correlation between clusters and sediment characteristics can be seen in Fig. 7. This was also shown in the ordination. Fig. 10B shows that the structural variable 'cluster' was highly significantly correlated with not only environmental variables but also with chlorophyll a.

The correlation analyses showed that the effect of sediment properties on the benthic microflora is not easily explained by any single sediment variable measured in this investigation, but the subjective variable 'sediment type' gave a good indication of the sediment characteristics. What the correlations actually show is the importance of exposure to wave action, a variable not in itself easy to measure, though this has been done by Muus (1968).

4. DISCUSSION

4.1. Chlorophyll a content

In investigations carried out on tidal flats and in estuaries the study area has comprised several km² (e.g. Cadée and Hegeman, 1977; Colijn and Nienhuis, 1978; Riznyk and Phinney, 1972; Amspoker and McIntire, 1978; de Jonge, in press). It is probable that variations in sediment characteristics and hydrological characteristics are greater in such areas than in the small non-tidal area I investigated. However, since one aim was to study the effect of variation in substrate type it was an advantage to concentrate on a small area and in this way avoid the influence of other variables such as salinity and temperature.

Measured physical properties of the sediment varied less than was expected from the visual impression (for instance no sediment could be characterized as muddy). In spite of this there were significant correlations between chlorophyll a content and the sediment variables but no single variable was highly significant. However, only a few variables were considered and no chemical variables such as pH, sulphide or nutrient concentrations were measured. The fact that highly significant correlations were obtained for variables such as the subjective sediment type and distance from shore, supports the hypothesis that the distribution of the microphytobenthos is affected by a combination of several factors brought about by the degree of exposure to wave action.

Sediment structure is mainly the result of exposure to water movements, and degree of exposure is certainly one of the factors controlling the microphytobenthic distribution (Grøntved, 1960, 1962; Amspoker, 1977; Cadée and Hegeman, 1977; Colijn and Nienhuis, 1978; van den Hoek et al., 1979; Colijn and Dijkema, 1981; de Jonge, in press). The continuous type of variation in the study area is obviously related to the exposure gradient. Microscope observations of fresh sediment confirmed this. Exposure to wave action restricts the number of protruding cells, colonies, motile forms and detritus floccules in the sediment. The floccules serve as a substrate for a dense diatom flora (cf. Admiraal and Peletier, 1980). The extremely high chlorophyll a values near the shore on transects D-D' and E-E', however, represent the patchy type of variation and can be related to some discontinuity in the sediment characteristics.

The nearshore discontinuity in the type IV sediment is probably connected with the changed chemical conditions in the sediment (oxygen depletion and sulphide formation) arising from the large amounts of decaying organic matter that accumulate. The presence of a rich microflora indicated that these conditions did not inhibit the growth of microbenthic algae. Admiraal and Peletier (1980) found high population densities for benthic diatoms in the presence of decomposing organic matter in the Ems-Dollart estuary and suggest the following causes: (1) detritus floccules are favourable substrates for diatoms; (2) stimu-

lation by release of inorganic and organic nutrients; (3) decimation of the fauna feeding on diatoms; (4) capacity of diatoms to tolerate extreme conditions brought about by organic pollution. The first cause at least applies here. The physico-chemical changes may also lead to a change in the diatom flora favouring species with a high degree of tolerance of sulphides and the capacity to use organic compounds (Admiraal and Peletier, 1979, 1980). The situation can also be compared with the sulphuretum described by Fenchel (1969) from the coast of Denmark. He describes the sulphuretum as a biotope with a diversified microfauna and numerous algae including diatoms.

The small-scale variation, often referred to as micro-patchiness, had a fairly low value ($CV=12-13\%$) and approached what could be considered as an error of estimation. However, similar variation has also been reported by Riznyk and Phinney (1972) and Colijn and Nienhuis (1978), among others. Higher values for small-scale variation, up to 50% (CV) have also been found (see Table in Taasen and Høisater, 1981). Because of the greater degree of mixing of sediment exposed to wave action as on the sandbank, one would have expected less variation in the clean sand (type I) than in the more poorly sorted sediment near the shore. However, this was not so in my samples, which were taken in the summer.

If we assume that the range of small-scale variation of chlorophyll a content is the same for the whole of the study area, the true intermediate-scale variation at the sampling occasion was about 30% (CV). The previously investigated seasonal variation of chlorophyll a content was 57% (CV) for a sampling area corresponding to points D3 to D6 (station 2 in Sundbäck, 1983a). If the horizontal variation for station 2 is slight as indicated in this investigation (Fig. 4), the intermediate-scale patchiness should not have markedly influenced the values for seasonal variation. However, the degree of variation may change with the season. If patchiness is more likely to occur during seasons when storms are less common (spring and summer, see Fig. 5 in Sundbäck, 1983a), the variation measured here would represent the widest variation and a more even distribution would occur during the rest of the year.

By comparing the chlorophyll a values of points D3-D6 with the mean value for type II sediment and the values for the previous summers, it can be concluded that the seasonal values for chlorophyll a may well represent the type II sediment, which was also the most widespread type in the bay.

The vertical distribtuion of chlorophyll a and pheopigment agrees with profiles from the Danish coast of Öresund (Gargas and Gargas, 1982; Fenchel and Straarup, 1971). Living diatom cells found deepest down in the sediment were araphideans, i.e. cells that cannot actively migrate into the photic zone of the sediment (cf. Rao and Lewin, 1976; Gargas and Gargas, 1982). This also agrees with observations made with epifluorescent light on material from a tidal flat in the Wadden Sea, Netherlands (Sundbäck and Cadée, unpublished).

4.2. Species composition of diatom assemblages

When dealing with cleaned diatom cells, there is always the risk of contamination by dead cells and allochthonous material (Thomas, 1979; Pryfogle and Loewe, 1979). The examination of the fresh material showed that there were more empty frustules near the shore, but the proportions of taxa seemed to be the same as for living material. This agrees with fluorescent-microscope observations on material from a tidal flat in the Wadden Sea (Sundbäck and Cadée, unpublished). On the tidal flat about 25-50% of the diatom cells were dead in the top 5 mm sediment (cf. correction factor of c. 0.75 used by Taasen and Høisaeter, 1981), but since living and dead cells occurred in the same relative proportions the inclusion of dead cells would not markedly affect the diatom analyses. The increased importance of *Nitzschia pusilla* and *Amphora* sp. in the near-shore dark sand (cluster 4) could not be an artifact since these species were also observed as dominants in the fresh material. However, the possibility of contamination by dead frustules still remains and thus the results have to be interpreted with caution, as in every other diatom analysis where no attempt to separate living and dead cells is made.

The diatom community in the study area was recognized as the *Fragilaria pinnata* - *Opephora martyi*-association previously described for sampling station 2 (Sundbäck, 1983b). The structural differences found for the different sediment types were quantitative rather than qualitative and continuous rather than patchy.

Distinct microbenthic floras have been found on different sediment types on tidal flats (Brockmann, 1950; Colijn and Koeman, 1975; Colijn and Nienhuis, 1978). In estuaries the benthic diatoms are generally distributed along environmental gradients (salinity, desiccation; e.g. McIntire, 1978), but patchy distribution related to sediment properties has also been observed (Amspoker and McIntire, 1978). In salt marshes elevation and canopy height of the grasses may create distinct diatom communities (Sullivan, 1982).

The phytosociological idea of distinct associations may hold for distinctly differing parts of a habitat, but is less suitable for describing the gradual variation within a habitat found here, in spite of the different sediment types. The main type of variation in the species composition of diatoms was a quantitative change that formed a continuum following the exposure gradient. However, there was a break in the continuum illustrated in the ordination (Fig. 8) created by the increase in relative abundance in particular of two small motile species, *Nitzschia pusilla* and *Amphora* sp. Degree of exposure to wave action is presumably the primary cause of the change in habitat arising from the accumulation of decomposing organic matter in a sheltered position. Thus the degree of exposure to wave movement may affect the habitat, either directly by controlling the number of unattached and protruding microalgae or indirectly by changing chemical conditions in the sediment, e.g. amount of oxygen present, nutrients and types of bacteria (cf. DeFelice and Lynts, 1978).

The high chlorophyll a values in the material from the type IV near-shore sediment indicate that the microalgae not only tolerate the conditions there but actually thrive. The question arises as to whether some of the species with increased relative abundance are species with a higher tolerance of sulphide.

Admiraal and Peletier (1979), for example, observed different degrees of tolerance for different intertidal diatom species but also suggested that a combination of different types of tolerance controls the distribution. For example they found unexpectedly high sulphide tolerances for mixed populations of diatoms from stations not influenced by organic pollution. In this investigation, sulphide concentration was not measured, and thus it is not known whether the concentrations were limiting for species with low tolerance. Very little is known about the environmental requirements of marine and brackish-water species of benthic diatoms (see Wolf, 1982) and there are no standard indicator species as used in limnology (e.g. Palmer, 1969; Lange-Bertalot, 1979). The genus *Nitzschia* is usually considered to be tolerant of organic pollution. However, Admiraal and Peletier (1979) found that *Nitzschia* spp. isolated from a mud flat were not particularly sulphide tolerant. On the other hand, they noted that *Navicula salinarum* was fairly tolerant. *N. cf. salinarum* was also present in many samples, mostly small individuals, but the relative abundance was not higher in sediment type IV near the shore.

If we exclude the possibility of the effect of sulphides, the possibility remains that the near-shore discontinuity in sediment type IV is only a result of especially sheltered conditions, permitting the presence of many motile and colony-forming species together with detritus floccules inhabited by diatoms.

Life forms attached to sand grains (epipsammic) made up about 2/3 of the diatom flora in the study area the rest being small motile forms. Large motile forms which are characteristic of tidal flats were not common (but were commoner in a nearby sheltered muddy bay). Thus my investigation can be compared with Amspoker's (1977) on Scripps Beach, California. He described the epipsammic flora of the beach as one with high diversity, low dominance and broadly distributed taxa which also applies to the flora in my investigation. The diatom flora of sediments has been described as a community with high diversity also by others (Amspoker and McIntire, 1978; DeFelice and Lynts, 1978). Cook and Whipple (1982) suggest that the

wide variability of the habitat is responsible for the high diversity. This investigation, as well as the previous seasonal study in the same area, strengthens the impression of high, and fairly stable, diversity in benthic diatom communities of shallow water. However, a slight decrease in diversity occurred near the shore. A decrease in diversity is usually interpreted as an indicator of stress such as from organic pollution (Pielou, 1975). Decrease in diversity has also been observed in benthic diatom communities under eutrophic conditions (Sullivan, 1976; van Raalte et al., 1976). However, there are also indications that diversity is a stable character and of little value as a stress indicator (Smith et al., 1982).

This investigation shows that the microphytobenthic biomass (chlorophyll a) in the study area varies by a factor of 5 (extreme values of nearshore type IV sediment excluded) and that this variation is related to sediment type. No primary production measurements were made, but according to the high correlation between chlorophyll a content and production values (Sundbäck, 1983a) the primary production may be assumed to have the same spatial distribution. Although the chlorophyll a values previously obtained in the same area (Sundbäck, 1983a) represent the average value for the whole study area (cf. 3.1.), some of the variation that has been interpreted as short-term variation may to some extent reflect spatial variation related to varying sampling points.

Cadée (1980) concluded that short-term temporal variation in microphytobenthos on tidal flats is not pronounced (compared with e.g. that in phytoplankton) but that spatial variation is large and numerous samples must be taken. Since sampling in non-tidal areas is more time-consuming the aim of the investigation must determine the sampling strategy. When a representative value for annual microphytobenthic primary production is required for a study area, e.g. for a carbon budget, it is sufficient to sample only a few times but a large number of samples that include all sediment types must be taken in the area (cf. Cadée and Hegeman, 1977). When temporal variation and controlling factors are to be investigated, the sampling position must be fixed and restricted, and the small-scale variation determined.

APPENDIX

Species list

- Achnanthes biasoletiana* (Kützing) Grunow
A. hauckiana Grunow
A. lemmermannii Hustedt
A. pseudosolea Simonsen
A. cf. tenera Hustedt
Amphora abludens Simonsen
A. coffeaeformis (Agardh) Kützing
A. coffeaeformis var. *perpusilla* Grunow
A. exigua Gregory
A. ovalis Kützing
A. tenerrima Aleem & Hustedt
A. sp. 1
Amphiprora alata (Ehrenberg) Kützing
Caloneis amphisbaena (Bory) Cleve
Catenula adhaerens Mereschkowsky
Cocconeis diminuta Pantocsek
C. pediculus Ehrenberg
C. placentula et var. Ehrenberg
C. scutellum Ehrenberg
C. scutellum var. *cocconeiformis* Rabenhorst
C. sp. 1
C. sp. 4
Diploneis didyma (Ehrenberg) Ehrenberg
Epithemia sorex Kützing
E. turgida (Ehrenberg) Kützing
Fragilaria construens var. *venter* (Ehrenberg) Grunow
F. pinnata Ehrenberg
F. virescens var. *subsalina* Grunow
F. sp. 1
Gomphonema pseudoexiguum Simonsen
Licmophora gracilis (Ehrenberg) Grunow
Mastogloia elliptica (Agardh) Cleve
M. exigua Lewis
M. pumila (Grunow) Cleve
Navicula amphipleuroides Hustedt
N. arenicola Grunow

- N. biskanteri* Hustedt
N. clementis Grunow
N. cruciloides Brockmann
N. crucifera Grunow
N. cryptocephala et var. Kützing
N. cryptolyra Brockmann
N. digitoradiata (Gregory) Ralfs
N. flenatica Grunow
N. florinae Möller
N. forcipata Greville
N. gracilis Ehrenberg
N. hansenii Möller
N. humerosa Brebisson
N. cf. incerta Grunow
N. marina Ralfs
N. nolens Simonsen
N. peregrina (Ehrenberg) Kützing
N. peregrina var. *meniscus* (Schumann) Grunow
N. phyllepta Kützing
N. rostellata Kützing
N. salinarum et var. Grunow
N. subinflata Grunow
N. sp. 1
N. sp. 3
N. sp. 5
Nitzschia amphibia Grunow
N. dissipata (Kützing) Grunow
N. frustulum var. *subsalina* Hustedt
N. pusilla Lange-Bertalot
N. valdestriata Aleem & Hustedt
Opehora martyi Heribaud
O. schulzii Brockmann
Pinnularia ambigua Cleve
Rhoicosphenia curvata (Kützing) Grunow
Rhopalodia gibberula (Ehrenberg) Müller
Stauroneis salina Wm. Smith
Synedra tabulata et var. (Agardh) Kützing
Tropidoneis lepidoptera (Gregory) Cleve

REFERENCES

- Admiraal, W. and Peletier, H. 1979: Sulphide tolerance of benthic diatoms in relation to their distribution in an estuary. - *Br. phycol. J.* 14: 185-196.
- Admiraal, W. and Peletier, H. 1980: Distribution of diatom species on an estuarine mudflat and experimental analysis of the selective effect of stress. - *J. Exper. Mar. Biol. Ecol.* 46: 157-175.
- Amspoker, M.C. 1977: The distribution of intertidal epipsammic diatoms on Scripps Beach, La Jolla, California, USA. - *Bot. Mar.* 20: 227-232.
- Amspoker, M.C. and McIntire, C.D. 1978: Distribution of intertidal diatoms associated with sediments in Yaquina Estuary, Oregon. - *J. Phycol.* 14: 387-395.
- Brockmann, G. 1950: Die Watt-Diatomeen der Schleswig-Holsteinischen Westküste. - *Abh. seckenb. naturf. Ges.* 478: 1-26.
- Cadée, G.C. 1980: Reappraisal of the production and import of organic carbon in the western Wadden Sea. - *Neth. J. Sea Res.* 14: 305-322.
- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. - *Neth. J. Sea Res.* 8: 260-291.
- Cadée, G.C. and Hegeman, J. 1977: Distribution of primary production of the benthic microflora and accumulation of organic matter on a tidal flat area, Balgzand, Dutch Wadden Sea. - *Neth. J. Sea Res.* 11: 24-41.
- Colijn, F. and Koeman, R. 1975: Das Mikrophytobenthos der Watten, Strände und Riffe um Hohen Knechtsand in der Wesermündung. - *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 26: 53-83.
- Colijn, F. and Nienhuis, H. 1978: The intertidal microphytobenthos of the 'Hohe Weg' shallows in the German Wadden Sea. - *Forschungsstelle Insel- und Küstenschutz Nordeney, Jahresbericht* 29: 149-174.
- Colijn, F. and Dijkema, K.S. 1981: Species distribution of benthic diatoms and distribution of chlorophyll a on an intertidal flat in the Dutch Wadden Sea. - *Mar. Ecol.* 4: 9-21.
- Cook, L.L. and Whipple, S.A. 1982: The distribution of edaphic diatoms along environmental gradients of a Louisiana salt marsh. - *J. Phycol.* 18: 64-71.
- DeFelice, D.R. and Lynts, G.W. 1978: Benthic marine diatom associations: Upper Florida Bay (Florida) and associated sounds. - *J. Phycol.* 14: 25-33.
- Dixon, W.J. (ed.) 1981: BMDP statistical software. - 726 pp., Berkeley.
- Edler, L. (ed.) 1979: Recommendations on methods for marine biological studies in the Baltic Sea. *Phytoplankton and chlorophyll*. - *BMB Publ.* 5, 38 pp.
- Fenchel, T. 1969: The ecology of marine microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated Protozoa. - *Ophelia* 6: 1-182.
- Fenchel, T. and Straarup, B.J. 1971: Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. - *Oikos* 22: 172-182.
- Gargas, M. and Gargas, E. 1982: Growth physiological conditions of marine microalgae in the topmost 10 cm of the sediment. - *Vatten* 38: 189-198.
- Gray, J.S. 1981: The ecology of marine sediments. 185 pp., Cambridge.

- Grøntved, J. 1960: On the productivity of microphytobenthos and phytoplankton in some Danish fjords. - Medd. Danm. Fiskeri- og Havsunders. 3: 55-92.
- Grøntved, J. 1962: Preliminary report on the productivity of microbenthos and phytoplankton in the Danish Wadden Sea. - Medd. Danm. Fiskeri- og Havsunders. 3: 347-372.
- Hoek, van den, C., Admiraal, W., Colijn, F. and de Jonge, V.N. 1979: The role of algae and seagrasses in the ecosystem of the Wadden Sea: A review. - In Wolff, W.J. (ed.) Flora and vegetation of the Wadden Sea: 3/1-206.
- Jansson, A.-M., Kautsky, N., von Oertzen, J.-A., Schramm, W., Sjöstedt, B., von Wachenfeldt, T. and Wallentinus, I. 1982: Structural and functional relationships in a southern Baltic *Fucus* ecosystem. - Contr. Askö Lab. 28, 95 pp.
- Johansson, C. 1982: Attached algal vegetation in running waters of Jämtland, Sweden. - Acta Phytogeogr. Suec. 71. 84 pp.
- Jonge, de, V.N. (in press): The occurrence of epipsammic diatom populations: a result of interaction between physical sorting of sediment and some properties of diatom species. - Est. Coast. Shelf Sci.
- Lange-Bertalot, H. 1979: Pollution tolerance of diatoms as a criterion for water quality estimation. - Nova Hedwigia Beih. 64: 285-304.
- Liljelund, L.-E. 1977: Diversitetsindex - en översikt (Diversity indices - a review). - Svensk Bot. Tidskr. 71: 165-176.
- Lorenzen, C.J. 1967: Determination of chlorophyll and pheopigments. Spectrophotometric equations. - Limnol. Oceanogr. 12: 343-346.
- Maarel, van der, E., Janssen, J.G.M. and Louppen, J.M.V. 1978: TABORD, a program for structuring phytosociological tables. - Vegetatio 38: 143-156.
- McIntire, C.D. 1978: The distribution of estuarine diatoms along environmental gradients: A canonical correlation. - Est. Coast. Mar. Sci. 6: 447-457.
- Muus, B.J. 1968: A field method for measuring "exposure" by means of plasterballs. - Sarsia 34: 61-68.
- Palmer, G.M. 1969: A compositive rating of algae tolerating organic pollution. - J. Phycol. 5: 78-82.
- Persson, S. 1977: Datorprogram för bearbetning av vegetationsdata. 1. Klassifikationsprogram - dokumentation och handhavande. - Meddn Avd. Ekol. Bot., Lunds Universitet 33. 68 pp.
- Persson, S. 1978: Datorprogram för bearbetning av vegetationsdata 2. Ordinationsprogram - dokumentation och handhavande. - Meddn Avd. Ekol. Bot., Lunds Universitet 34. 64 pp.
- Persson, S. 1980: Succession in a south Swedish dediduous wood: a numerical approach. - Vegetatio 43: 103-122.
- Pielou, E.C. 1975: Ecological diversity. 165 pp. New York.
- Pryfogle, P.A. and Loewe, R.L. 1979: Sampling and interpretation of epilithic lotic diatom communities. - In Weitzel (ed.): Methods and measurements of periphyton communities pp. 77-89.
- Raalte, van, C.D., Valiela, I. and Teal, J.M. 1976: The effect of fertilization on the species composition of salt marsh diatoms. - Water Res. 10: 1-4.
- Rao, V.N.R. and Lewin, J. 1976: Benthic marine diatom flora of False Bay, San Juan Island, Washington. - Syesis 9: 173-213.
- Riznyk, R.Z. and Phinney, H.K. 1972: The distribution of intertidal phytoplankton in an Oregon estuary. - Mar. Biol. 13: 318-324.
- Roskam, E. 1971: Program ORDINA: Multidimensional ordination of observation vectors. - Programme-Bull. 16. Dept. of Psychology, University of Nijmegen. 8 pp.

- Smith, W., Gibson, V.R., Brown-Leger, L.S. and Grassle, J.F. 1979: Diversity as an indicator of pollution: Cautionary results from microcosm experiments. - In Grassle, J.F. et al. (eds.): Ecological diversity in theory and practice, pp. 269-277.
- Sullivan, M.J. 1976: Long-term effects of manipulating light intensity and nutrient enrichment on the structure of a salt marsh diatom community. - J. Phycol. 12: 205-210.
- Sullivan, M.J. 1982: Distribution of edaphic diatoms in a Mississippi salt marsh: A canonical correlation analysis. - J. Phycol. 18: 130-133.
- Sundbäck, K. 1983a: Seasonal variation in microphytobenthic primary production and chlorophyll *a* content on a sandy coast with shallow brackish water, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. 1983b: The species composition of benthic diatom assemblages on a sandy coast with shallow brackish water, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Taasen, J.P. and Høisaeter, T. 1981: The shallow-water soft-bottom benthos in Lindåspollene, Western Norway. 4. Benthic marine diatoms, seasonal density fluctuations. - Sarsia 66: 293-316.
- Thomas, D.P. 1979: Some problems connected with use of cleaned cells and relative proportions of species in diatom ecology.- Nova Hedwigia, Beiheft 64: 503-511.
- Wachenfeldt, von, T. 1975: Marine benthic algae and environment in the Öresund. - Doct. thesis, 328 pp. University of Lund.
- Wolf, de, H. 1982: Method of coding of ecological data from diatoms for computer utilization. - Med. Rijks Geol. Dients 36: 95-98.
- Öresundskommissionen 1980: Öresund. Tillstånd - effekter av närsalter. - 252 pp. Stockholm.

THE RESPONSE OF PLANKTONIC AND MICROBENTHIC
ALGAL ASSEMBLAGES TO NUTRIENT ENRICHMENT IN
SHALLOW COASTAL WATERS, SW SWEDEN

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ABSTRACT

Nutrient enrichment experiments (N and P) were performed both in the field and in the laboratory using natural phytoplankton and microphytobenthos assemblages of shallow water in Lomma Bay, which is heavily polluted with inorganic nutrients, and in the basin at the southern entrance to the Falsterbo canal which has low nutrient concentrations.

Both phytoplankton and microphytobenthos from the Falsterbo canal increased their biomass after the addition of nitrogen. Phytoplankton growth was stimulated by the addition of phosphorus to nitrogen-rich water from the polluted Lomma Bay. The microphytobenthos showed no increase in biomass after the addition of nutrients. The rate of decrease in inorganic nitrogen in the water was higher in containers with sediment than in those containing phytoplankton. We estimated that the uptake of nitrogen by the microphytobenthos accounted for c. 20% of the decrease in inorganic nitrogen from the water. The rest was lost in other ways, e.g. by denitrification.

When containers with microphytobenthos from Lomma Bay were kept in the dark phosphorus was released at a rate of up to c. $180 \mu\text{mol m}^{-2} \text{ day}^{-1}$. We suggest that by producing oxygen microbenthic algae keep the sediment surface oxygenated thereby decreasing phosphorus transport from the sediment to the overlying water.

INTRODUCTION

Phytoplankton in coastal waters is often limited (in the sense of Liebig) by nitrogen (Goldman, 1976). Little work has been done on microphytobenthos and nutrient limitation, as it has been assumed that these algae have access to an inexhaustible nutrient supply from the interstitial water of the sediments where they live (Cadée and Hegeman, 1974; van den Hoek et al., 1979).

Nutrient enrichment experiments in salt marshes have given varying results: Changes in microflora diversity (van Raalte et al., 1976a); stimulation of growth (Darley et al., 1981); no effect at all (Sullivan, 1981).

Nitrogen additions to the surface water have been shown to stimulate phytoplankton production on the SW coast of Sweden (Granéli, 1978, 1981; Granéli et al., in press). Although work on microphytobenthos has been done in Öresund (The Sound) (Gargas, 1970; Gargas and Gargas, 1982; Sundbäck and Persson, 1981; Sundbäck, 1983a, b, c), nutrient/microphytobenthos relations have not been investigated. The coasts of Öresund are mainly sandy with shallow water and the microphytobenthos contributes significantly to the total primary production (Sundbäck, 1983a).

Our experiments were designed to find answers to the following questions:

- (1) Are the phytoplankton and microphytobenthos stimulated by the addition of the same nutrient?
- (2) Is the rate of biomass increase the same for the phytoplankton and the microphytobenthos when a limiting nutrient is added?
- (3) Do the phytoplankton and microphytobenthos react in the same way in a eutrophicated area and an area not affected by discharge?
- (4) How much of the added nutrients (phosphorus and nitrogen) becomes incorporated into the microalgae and how much is converted by bacterial and chemical processes?

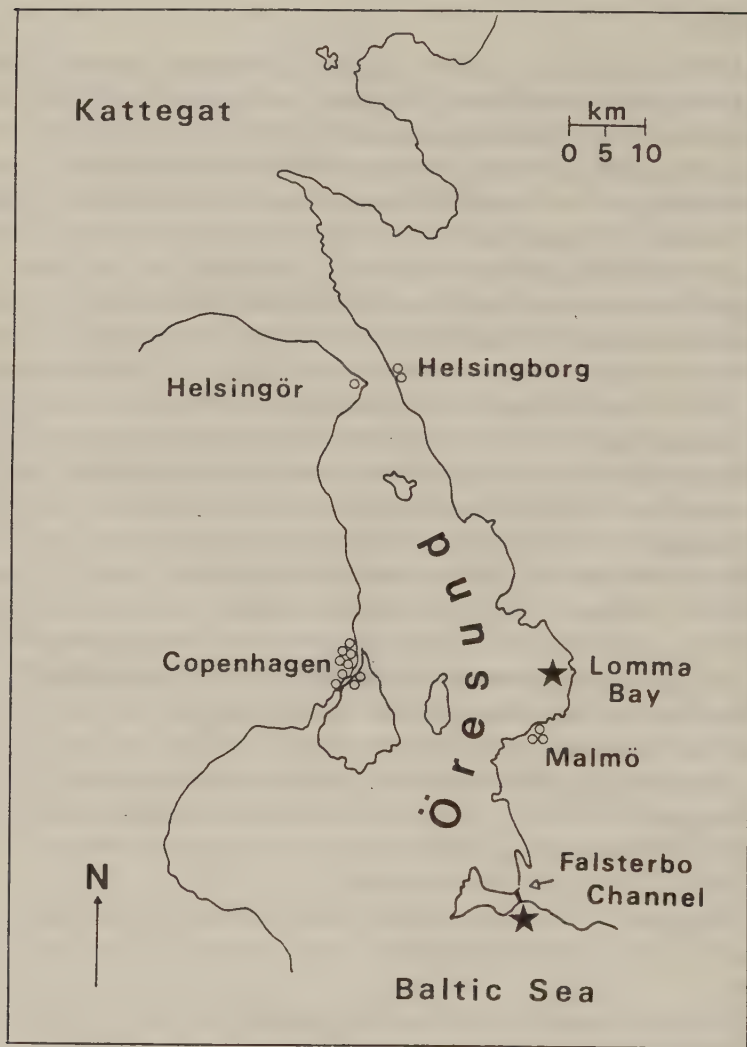


Fig. 1. Öresund showing experimental and sampling sites (★).

MATERIAL AND METHODS

Four experiments were run: two field experiments (A and B) and two laboratory experiments (C and D). In each of the four experiments a series of containers with unfiltered seawater (=phytoplankton containers) were used and a series of containers with sediment and filtered seawater (=sediment containers). For the different types of containers used see Experimental design under Field experiments and Laboratory experiments.

Microalgae were collected from the basin at the southern entrance to the Falsterbo canal and from Lomma Bay, both on the SW coast of Sweden (Fig. 1), in shallow water (0.5--2 m) with sandy bottoms. The Falsterbo canal is not directly influenced by sewage whereas Lomma Bay receives a heavy load of municipal sewage from a tertiary treatment plant (phosphorus removal) belonging to Malmö (population c. 300,000).

Field experiments (A and B)

(a) Experimental design

These experiments were performed in the basin. Experiment A ran from August 23 to 28, 1978 (but the plankton was sampled for 6 additional days), Experiment B ran from July 1 to 7, 1979. See Granéli (1981) for investigation of the phytoplankton. In brief: A series of 200-litre polythene bags hung from a wooden frame were filled with 150 l of unfiltered seawater containing indigenous plankton assemblages.

The microphytobenthos was investigated in two different ways: In Experiment A we used cylinders constructed of PVC frames (ID 0.4 m, length 1 m) covered (except at the bottom) with a double polythene sheet (0.1 mm thick). The open end was pressed down into the sediment to a depth of 20 cm (Fig. 2). In Experiment B the top 1 cm of the sediment was collected from the same site as Experiment A, sieved (0.5 mm mesh size) and transferred to cylindrical plexiglass aquaria (volume 0.8 l). The aquaria, containing a 5 cm layer of sediment and 300 ml of filtered seawater (Whatman GF/F glass fibre filter), were incubated outdoors and kept cool with water from the canal.

Fig. 2. PVC frame used for investigating the microphytobenthos in situ (Experiment A).

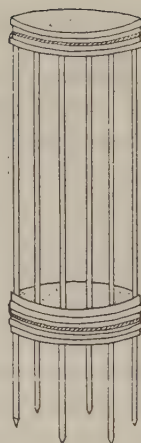


Table 1. Initial concentrations and daily additions of inorganic phosphorus and nitrogen to the water in the field experiments (A and B).

	Initial conc. of seawater		Nutrients added			
	<u>Expt A</u>	<u>Expt B</u>	<u>Expt A</u>	<u>Expt B</u>		
				Phytopl.	Sed.	
PO ₄ -P, ·10 ⁻⁶ M	0.26	0.25	Initial	0.77	1.13	11.3
			Daily	0.07	0.35	0.9
NO ₃ -N, ·10 ⁻⁶ M	1.3	0.8	Initial	28.6	25	250
			Daily	1.1	11	28.6

In both Experiments A and B the water in all containers was enriched with the following nutrients: phosphorus added as KH₂PO₄, nitrogen added as NaNO₃ and both added together. Containers with no added nutrients served as controls (C). Duplicate aquaria were used for the microphytobenthos in Experiment B. Table 1 shows the initial concentrations and daily additions of nutrients.

(b) Sampling procedure

Phytoplankton: In Experiment A 20 l of the water were removed from each bag every day after thoroughly mixing the water. 20 l of unfiltered seawater were then added together with nutrients. In Experiment B 5 l of the water were removed from each bag daily, but only nutrients were added (Granéli, 1981).

Microphytobenthos: In Experiment A the top 5 mm of the sediment was sampled daily with a plexiglass corer (2 cm ID) (Sundbäck, 1983a). Four sediment samples and a 500 ml sample of the water for nutrient analysis were taken from each container. The water was carefully mixed before sampling and again after the addition of nutrients. In Experiment B, 150--200 ml of water were removed daily for nutrient analysis and replaced with the same amount of filtered seawater to which nutrients had been added. Sediment samples for measuring chlorophyll *a* content and primary production were taken with a cut-off 2 ml plastic syringe (ID 0.9 cm). The top 0.5 cm of the sediment was sampled.

Laboratory experiments (C and D)

Experiment C, with water and sediment from Lomma Bay, ran for 10 days from September 28 to October 7, 1979. Experiment D, with material from the Falsterbo canal, ran from October 19 to November 10, 1981 (23 days).

Surface water was collected directly into 5-litre plastic containers where the water was 0.5 m deep. The top 1 cm of sediment was collected at the same depth and all but a small portion was sieved (mesh size 0.5 mm) to remove the macrofauna.

(a) Experimental design

Seawater for the phytoplankton assays was filtered through a 150 μ m nylon net to remove large zooplankton and poured into 4-litre spherical flasks. The microphytobenthos was investigated in aquaria each with a 3--4 cm layer of homogenized sediment and with filtered seawater. In Experiment C we used

cylindrical 0.8-litre plexiglass aquaria (cf. Experiment B) with sediment and 500 ml of seawater. In Experiment D 8-litre rectangular plastic aquaria with sediment and 5 l of seawater were used. Nutrients (P, N and PN) were added at the beginning of each experiment only (c.f. field expts) (Table 2). Note: since the aquaria used in Experiment C were small, the 400 ml of water removed for analysis had to be replaced each time and the nutrients replaced: $1.8 \cdot 10^{-6}$ M $\text{PO}_4\text{-P}$ and $304 \cdot 10^{-6}$ M $\text{NO}_3\text{-N}$.

In the microphytobenthos assays two additional containers with added PN were used: One with sieved sediment kept in the dark (DARK), to estimate the nutrient uptake in the dark and one with unsieved sediment (UNSIEVED), to get a rough idea of the importance of bioturbation in the flux of nutrients between sediment and water.

Both experiments were run in a 16/8 h light/dark cycle. The temperature in Experiment C was $20 \pm 1^\circ\text{C}$, and $13 \pm 1^\circ\text{C}$ in Experiment D. The two laboratory experiments also differed as to duration, sampling intervals, amount of nutrients added and the initial nutrient concentration of the seawater (Table 2).

Table 2. Laboratory experiment (C and D). Initial concentration of seawater and amounts of inorganic phosphorus and nitrogen added to the water at the beginning of the experiments.

	<u>Initial conc.</u> <u>of seawater</u>		<u>Nutrients added</u>	
	Expt C	Expt D	Expt C	Expt D
$\text{PO}_4\text{-P}, \cdot 10^{-6}\text{M}$	2.1	0.8	1.8	17.7
$\text{NO}_3\text{-N}, \cdot 10^{-6}\text{M}$	179.0	3.5	60.7*	607.1

* From the third day onwards $304 \cdot 10^{-6}\text{M}$ of N was added to the containers with sediment.

(b) Sampling procedure

500 ml aliquots were taken from the phytoplankton containers for measuring nutrients, chlorophyll a content and primary production. The water was not replaced. In Experiment C 400 ml of water were taken from each sediment container for nutrient analysis and replaced with filtered seawater (see above). Sediment samples for measuring chlorophyll a content and primary production were taken in the same way as in Experiment B.

In Experiment C samples were taken every second day and in Experiment D at 3- to 5-day intervals.

Analysis (all four experiments)

Uptake rates were obtained by measuring changes in phosphorus and nitrogen concentrations in the water. In addition the nutrient concentrations of the interstitial water were measured at the beginning and end of Experiment D. The inorganic nutrients ($\text{PO}_4\text{-P}$, $\text{NH}_4\text{-N}$, $\text{NO}_3 + \text{NO}_2\text{-N}$) were analysed immediately after sampling according to methods described in Carlberg (1972).

The response of the microalgae to the addition of nutrients was obtained by measuring changes in chlorophyll a content and primary production rates in the water and in the sediment. For phytoplankton chlorophyll a measurements the water was filtered through Whatman GF/F filters and the chlorophyll a content was determined according to Jeffrey and Humphrey (1975). Phytoplankton primary production was estimated in terms of ^{14}C uptake. 30 ml glass bottles were incubated in situ (field experiments) or in an incubation chamber (laboratory experiments). For further details see Granéli (1981).

The chlorophyll a content of the microphytobenthos was measured after extraction with 90% acetone as described in Lorenzen (1967). Primary production of the sediment was measured by ^{14}C uptake according to Gargas (1970). Incubation was as for phytoplankton. For further details see Sundbäck (1983a).

RESULTS

Field experiments (A and B)

Phytoplankton: Additions of nitrogen stimulated the growth of phytoplankton while the addition of phosphorus alone gave no response (Fig. 3). Additions of nitrogen alone gave maximal chlorophyll a values of 2.4 and 3.6 times the values in control containers in Expts A and B respectively. Nitrogen and phosphorus added together gave chlorophyll a values of 3.7 and 11 times the control values in Experiments A and B respectively. Primary production showed the same trend as chlorophyll a content in both experiments (Fig. 3). For further details see Granéli (1981).

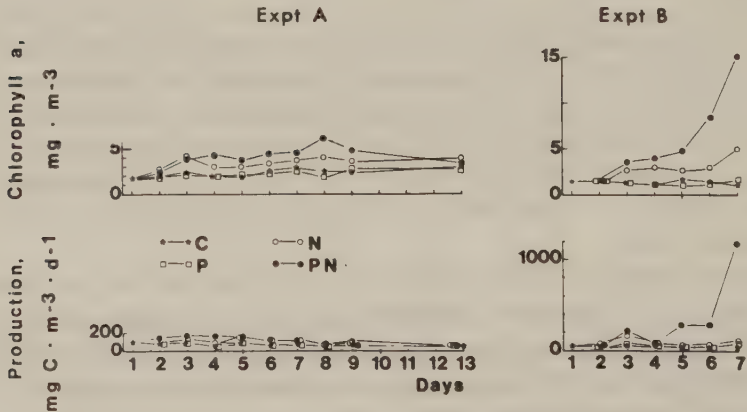


Fig. 3. Chlorophyll a content and primary production in the phytoplankton containers of the field experiments (A and B) in the basin of the Falsterbo canal. After Granéli (1981).

Microphytobenthos: In Experiment A the chlorophyll a values showed no clear trend and after a slight increase on the second day primary production decreased in all sediment containers (Fig. 4). The containers with added P and N were damaged on the fifth day by fluctuations in water level and were of no further use.

In experiment B the chlorophyll a values increased until the third or fourth day (Fig. 4). The addition of P did not stimulate the chlorophyll production to above the control values. The addition of N increased the values slightly and N and P added together had the strongest effect, but the increase was only 10% above the control values (Fig. 4). The ^{14}C uptake remained at the initial level during the first two days, decreased between the third and fourth day (cf. phytoplankton) and increased thereafter until the end of the experiment (Fig.4).

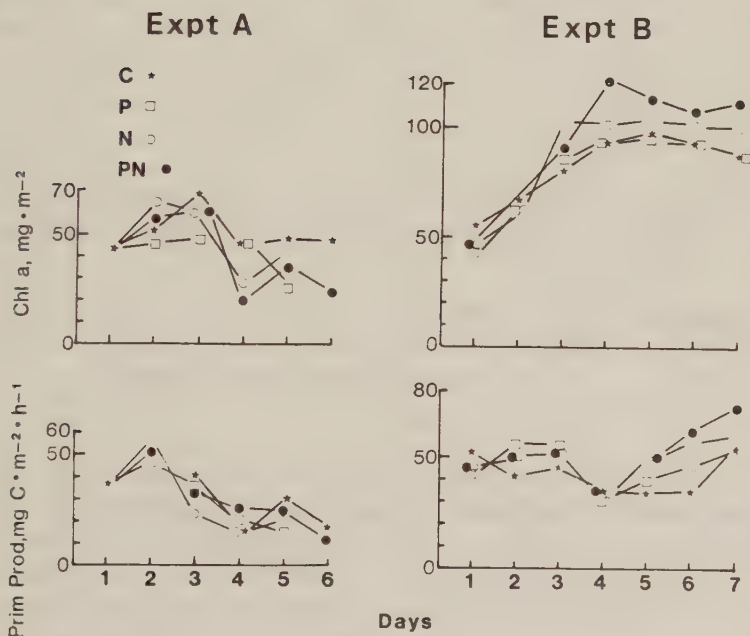


Fig. 4. Chlorophyll a content and primary production in the sediment aquaria of the outdoor experiments (A and B) (in the Falsterbo canal).

Laboratory experiments (C and D)

Chlorophyll a content, primary production and assimilation number: Water from Lomma Bay (Experiment C) supported a much higher microalgal biomass than water from the Falsterbo canal (Experiment D): the chlorophyll a values in the control containers were 40 times higher for the phytoplankton and 4 times higher for the sediment (Table 3, Figs. 5 and 6).

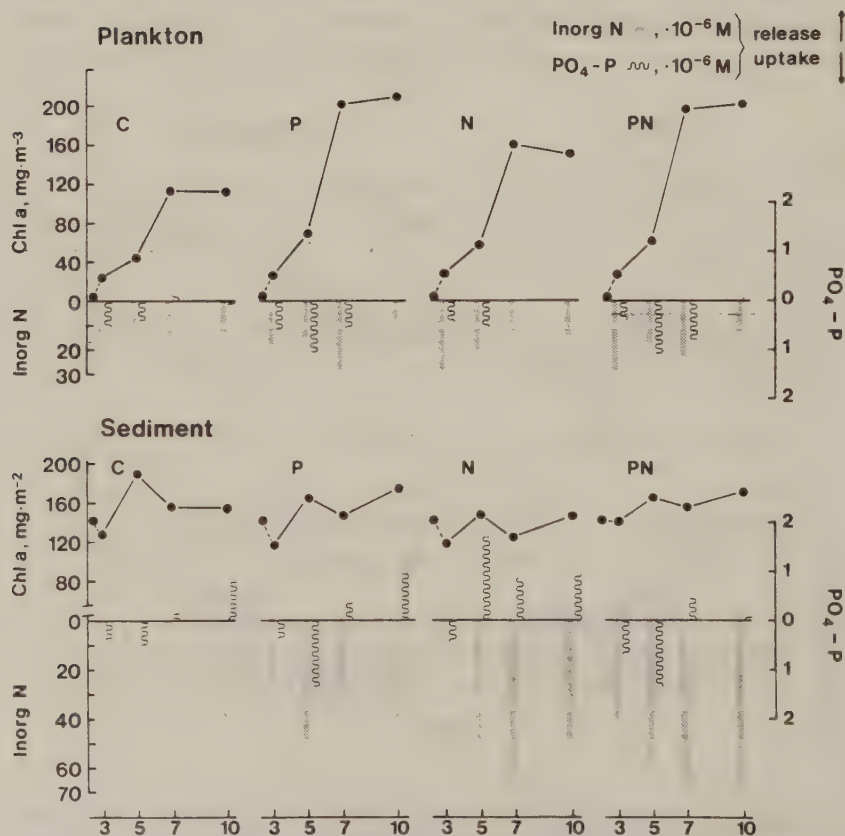


Fig. 5. Chlorophyll a content and rate of uptake of inorganic phosphorus and nitrogen in laboratory experiment C (Lomma Bay). C = control, P = added phosphorus, N = added nitrogen and PN = phosphorus and nitrogen added together.

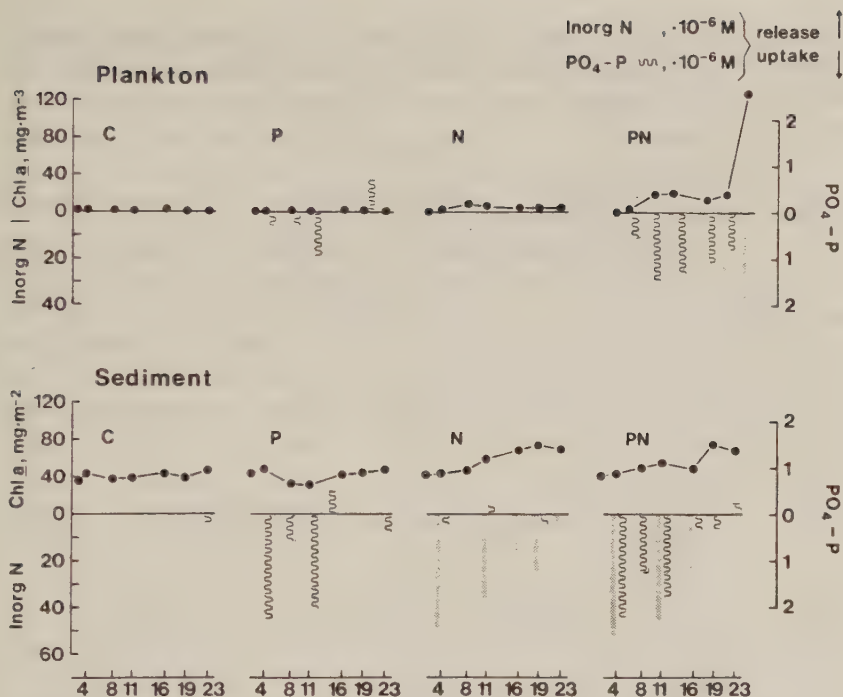


Fig. 6. Chlorophyll a content and rate of uptake of inorganic phosphorus and nitrogen in laboratory experiment D (Falsterbo canal). For experimental design see Fig. 5.

In the Lomma Bay experiment (C) the addition of P stimulated the phytoplankton chlorophyll a production more than the addition of N (Fig. 5). P added alone had the same effect as when added together with N. Nutrient additions had, however, no effect on the chlorophyll a content of the sediment.

In the Falsterbo canal experiment (D), however, P alone gave no stimulance and a depression in the phytoplankton chlorophyll a production was actually observed (Fig. 6). N added alone stimulated both phytoplankton and microphytobenthic growth and when it was added together with P phytoplankton chlorophyll a increased remarkably. The difference in the response of the microphytobenthos to the addition of N and of PN was less marked (Fig. 6).

Table 3. Maximum values for chlorophyll a content, primary production and assimilation number in the different containers in the laboratory experiments C (Lomma Bay) and D (Falsterbo canal).

	Chlorophyll <u>a</u>		Primary production		Assimilation number	
	<u>Plankton</u>	<u>Sediment</u>	<u>Plankton</u>	<u>Sediment</u>	<u>Plankton</u>	<u>Sediment</u>
	mg m ⁻³	mg m ⁻²	mg C m ⁻³ h ⁻¹	mg C m ⁻² h ⁻¹	mg C mg chl <u>a</u> ⁻¹ h ⁻¹	mg C mg chl <u>a</u> ⁻¹ h ⁻¹
<u>Exp. C</u>						
Initial	4.6	143.1	6.6	120.8	1.42	0.84
C max	112.7	187.6	320.0	150.4	2.84	1.21
P max	208.0	172.7	645.0	119.8	3.22	1.05
N max	159.7	146.1	428.3	104.5	2.68	1.12
PN max	200.0	169.5	645.0	101.1	3.30	0.73
<u>Exp. D</u>						
Initial	1.6	38.3	4.4	50.7	2.84	1.33
C max	2.8	47.1	8.9	54.4	4.38	1.46
P max	2.3	47.7	14.1	53.5	2.85	1.65
N max	9.1	74.3	33.0	98.2	5.41	1.72
PN max	127.8	75.6	368.3	242.4	10.02	3.21

Primary production in both phytoplankton and microphytobenthos followed the same trend as chlorophyll a production in both laboratory experiments. In the sediment, however, the response of primary production to the PN addition was much stronger than for chlorophyll a production in Expt D (Table 3).

Assimilation number: The assimilation numbers (mg C · mg chl a⁻¹ · h⁻¹) were higher in Experiment D than in C and higher for phytoplankton than for microphytobenthos. The highest values were obtained in the containers with added P and N in Experiment D, i.e. 10 mg C · mg chl a⁻¹ · h⁻¹ for phytoplankton and 3.2 mg C · mg chl a⁻¹ · h⁻¹ for microphytobenthos (Table 3).

Uptake and release of nutrients: The concentration of inorganic nutrients was high in the water from Lomma Bay ($2.1 \cdot 10^{-6} \text{ M P}$ and $179 \cdot 10^{-6} \text{ M N}$). The nutrient content of the water from the Falsterbo canal was on the other hand low ($0.8 \cdot 10^{-6} \text{ M}$ of P and $3.5 \cdot 10^{-6} \text{ M}$ of N). The difference in initial nutrient concentrations was reflected in the different uptake rates of P and N in the two experiments.

The concentrations of inorganic P and N decreased more rapidly in the sediment containers than in the phytoplankton containers. Maximum P uptake by phytoplankton was $1.4 \cdot 10^{-6} \text{ M day}^{-1}$ and N uptake $46 \cdot 10^{-6} \text{ M day}^{-1}$ (containers with PN in Experiment D) (Fig. 6). Corresponding maximum values for the

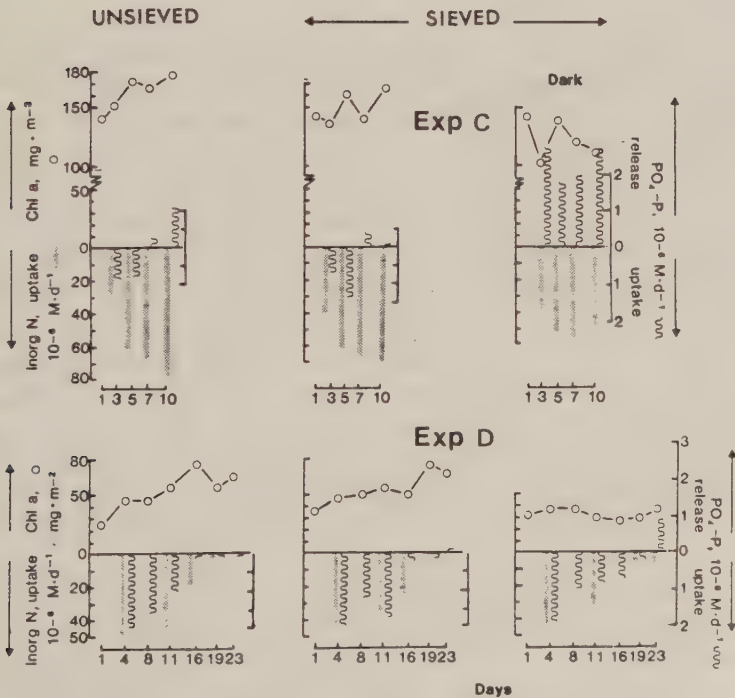


Fig. 7. Chlorophyll a content and uptake rate of N and P in sediment containers with addition of both phosphorus and nitrogen under varying conditions. For experimental design see Material and Methods.

sediment were for P $2.2 \cdot 10^{-6} \text{ M day}^{-1}$ or $185 \mu\text{mol m}^{-2} \text{ day}^{-1}$ (containers with P in Experiment D) and for N $80 \cdot 10^{-6} \text{ M day}^{-1}$ or $5.1 \text{ mmol m}^{-2} \text{ mmol m}^{-2} \text{ day}^{-1}$ (UNSIEVED, Experiment C) (Fig. 7).

As a rule the highest uptake rates of N and P in both experiments were found in the containers with P + N for both phytoplankton and sediment. In the control containers and containers with P or N the uptake rates, however, were not the same in Experiment C and Experiment D. In the C and P containers, with the initially nitrogen-rich Lomma water (Experiment C), N uptake was high, especially in the sediment containers (cf. Figs 5 and 6). In Experiment C P was used up in the phytoplankton containers but in the sediment containers P was released from the sediment at rates of up to $1.6 \cdot 10^{-6} \text{ M day}^{-1}$ (c. $100 \mu\text{mol m}^{-2} \text{ day}^{-1}$) (Fig. 5).

The initial chlorophyll a content of the sediment was much higher in Experiment C than in D: 140 mg m^{-2} compared with 35 mg m^{-2} (sieved) and 25 mg m^{-2} (unsieved). Nevertheless, chlorophyll a content increased towards the end of both experiments. Note that Experiment C ran for only 10 days but Experiment D for 23 days (Fig. 7). In the sediment containers kept in the dark the chlorophyll a content decreased somewhat in Experiment C and remained at the same level in Experiment D.

In both experiments P and N were taken up in about the same proportions by sieved and unsieved sediment in containers kept in the light (Fig. 7). In Experiment C, P was released after 5 days in both containers kept in the light. Uptake of N was slightly less in the dark than in the light, but higher uptake rates in the dark were observed a few times in Experiment D. P, on the other hand, was released at rates of up to $2.8 \cdot 10^{-6} \text{ M day}^{-1}$ or $178 \mu\text{mol m}^{-2} \text{ day}^{-1}$ in the Lomma sediment, probably because anoxic conditions developed. In Experiment D, however, P was released only towards the end of the experiment (Fig. 6).

In Experiment D the initial values of N and P in the interstitial water were $155 \cdot 10^{-6} \text{ M}$ or 2.5 mmol m^{-2} (water comprised 40% of the sediment volume) and $13 \cdot 10^{-6} \text{ M}$ ($=0.2 \text{ mmol m}^{-2}$) respectively. The N concentration of the interstitial water decreased by 8 to 50 times during the experiment in the containers with sieved sediment kept in the light. P concentrations in the interstitial water on the other hand increased (up to 8 times) during the experiment.

DISCUSSION

The results show that both phytoplankton and microphytobenthos are nitrogen-limited in the Falsterbo area. The phytoplankton, however, responded with a much higher increase in biomass (c. 80 times the initial value) than the microphytobenthos (c. 2 times the initial value) and built up high chlorophyll a concentrations from initially low levels (Table 3). The slower increase in microphytobenthic biomass can be explained in two ways: (1) the biomass of microphytobenthos is initially high because of adequate nutrient conditions and (2) the microphytobenthic algae receive light only through the sediment so that light becomes the factor that is most limiting, not nutrient availability.

Diatoms were the main constituents of both florae, but while planktonic diatoms may divide up to 4 times a day, benthic diatoms may only divide 0.3 times a day (Admiraal et al., 1982).

Phytoplankton was nitrogen-limited in all experiments using water from Falsterbo, which is in agreement with previous experiments in this area (Granéli, 1978, 1981). Microphytobenthos may also be limited by N, as was the case in two of the three experiments (B and D). In the third experiment (C, Fig. 5) the added nutrient concentration was probably too low to stimulate the growth of flora adapted to high nutrient concentrations.

Additions of nutrients have been found to increase chlorophyll a content and primary production of edaphic algae in salt marshes (Darley et al., 1981; van Raalte et al., 1976b; Sullivan and Daiber, 1975). No direct effect was noticed by Estrada et al. (1974). On tidal flats even deleterious effects on benthic diatoms have been observed after large additions of ammonium in combination with high light intensity (Admiraal, 1977).

Sediment and water from the Falsterbo canal did not support high biomasses, neither of phytoplankton nor microbenthos, in contrast to sediment and water from the nutrient-rich Lomma Bay.

Darley et al. (1981) transplanted sediment cores with edaphic algae from a nutrient-rich salt marsh (creek bank) to a nutrient-poor *Spartina* marsh and found that after depletion of the initial nitrogen in the creek-bank cores the

water in the *Spartina* marsh was not able to sustain the algal biomass at the same level. When sediment cores were transplanted from the nutrient-poor to nutrient-rich marsh the local input of ammonium cancelled out nitrogen limitation in the *Spartina*-marsh algae and biomass increased.

Water from Lomma Bay, which contained high concentrations of inorganic nitrogen (mostly as nitrate), stimulated phytoplankton growth markedly, and when P was added as well an even higher biomass was obtained.

Phosphorus limitation in coastal waters is usually found in places where fresh-water inflow is high as in Trondheim Fjord (Sakshaug et al., 1983) and in the inner part of the Bay of Marseilles (Berland et al., 1980). In Laholm Bay (Sweden) the discharge of fresh water from rivers is very high (Fleischer et al., 1982), N, however, was shown to be the limiting nutrient all year round (Granéli and Granéli, in press; Granéli et al., in press). Phosphorus limitation in Lomma Bay is caused by discharge of tertiary-treated sewage with a high N/P ratio.

The microphytobenthos from Lomma Bay showed only a slight increase during the 11-day experiment (C). Why were these algae not stimulated by the high amounts of nutrients available?

Sullivan and Daiber (1975) found chlorophyll *a* values of up to 280 mg m^{-2} in the top 1 cm of sediment after the addition of nutrients to a Delaware salt marsh. Our values reached a maximum of 188 mg m^{-2} in the top 0.5 cm of sediment. This may be the maximum value that the benthic microalgae can reach in Lomma Bay. Chlorophyll *a* values of up to 400 mg m^{-2} in the top 1 cm of sediment have been reported for a bay on the Danish coast of Øresund (Gargas and Gargas, 1982).

Light was shown by Estrada et al. (1974) to be more important than nutrient availability for stimulating the growth of edaphic algae. Light rapidly becomes attenuated in the sediment, the photic zone being only a few mm (Fenchel and Straarup, 1971; Haardt and Nielsen, 1980).

Assimilation numbers were much higher for the phytoplankton than for the microphytobenthos. Low values were also found for the microphytobenthos by Darley et al. (1981) and the highest numbers (up to 0.96) were found in enriched cores.

Thomas (1970) suggested $P_{\max}$ values (carbon assimilation per unit chlorophyll a at light saturation, $\text{mg C mg chl } a^{-1} \text{ h}^{-1}$) of 3.15 in nitrogen-depleted waters and 4.95 in nitrogen-rich areas in tropical regions of the Pacific Ocean. For phytoplankton we found values of up to 4.38 for the controls and 5.41 (N) and 10.02 (PN) for the enriched containers. The microphytobenthic values ranged between 0.84 and 3.21.

The disappearance of nutrients from the water column in the euphotic zone is usually considered to be the result of incorporation into phytoplankton cells. At the sediment surface other factors such as bacterial activity may be more important. In shallow waters microphytobenthos may also play an important part in the uptake of nutrients. The processes of denitrification, nitrification and ammonification make it difficult to evaluate the N uptake of algae.

The interstitial water is generally considered to be the most important source of nutrients for microbenthic algae (e.g. van den Hoek et al., 1979). Phytoplankton on the other hand usually depends on rapid remineralization in the euphotic zone to maintain a high level of production in coastal waters (Harrison, 1977).

The decrease in rates of P and N in the phytoplankton containers reached values of c. $1.5 \cdot 10^{-6} \text{ M day}^{-1}$ for P and c. $40 \cdot 10^{-6} \text{ M day}^{-1}$ for N.

Nakajima et al. (1981), working in an experimental pond simulated a plankton bloom by adding P and N and found uptake rates of up to $143 \cdot 10^{-6} \text{ M day}^{-1}$ for N and $9 \cdot 10^{-6} \text{ M day}^{-1}$ for P. These values were similar to ours, while bearing in mind the fact that their chlorophyll a values were three times higher than in our experiments.

In Experiment D about 90% of the initial pool of N disappeared during the 23 days in the phytoplankton containers with N additions. The total fixation of carbon by phytoplankton during this experiment was about 45 g C m^{-3} . This production corresponds to an N uptake of $574 \cdot 10^{-6} \text{ M}$ using the Redfield C:N ratio 6.6 (by atoms) (Fig. 8). This value agrees well with the net disappearance of N ($559 \cdot 10^{-6} \text{ M}$).

The net decrease in N from the water column in the sediment containers was higher than in the phytoplankton containers;

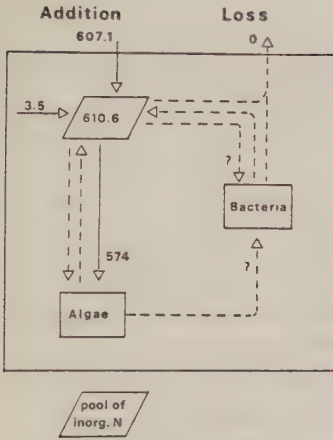
99.8% of the initial concentration of N in the water disappeared during Experiment D.

If we assume that the uptake of N from the water column by autotrophic organisms in the sediment is equal to the difference between the uptake in light and dark (see Fig. 7), the microphytobenthos would account for about 20% of the total decrease of N in the water. This value does not, however, tell us anything about the possible uptake from the interstitial water.

In Experiment D (23 days) the nutrients in the interstitial water were measured at the beginning and end of the experiment. We can therefore try to roughly calculate the uptake of N by the microphytobenthos from the total initial pool of inorganic N (overlying water + interstitial water) during the experiment. The initial pool of inorganic N in the container with P + N additions was $765 \cdot 10^{-6} \text{M}$ or 55.6 mmol m^{-2} (Fig. 8). At the end of the experiment c. 0.4 mmol m^{-2} remained i.e. 55.2 mmol m^{-2} (=99%) had been lost. In the dark less N was lost, i.e. 34 mmol m^{-2} (=61%). If we assume that the difference between the two amounts represents uptake by microbenthic algae in light, then this uptake is equal to 38% of the total net decrease in N or 21 mmol m^{-2} . The total microbenthic primary production during Experiment D was about 8.5 g C m^{-2} (a factor of 0.2 was used to correct for overestimation in the incubation method; see Sundbäck, 1983a). If we use the C:N ratio 22:1 (by atoms) for marine benthic plants (Atkinson and Smith, 1983) this production would correspond to $32 \text{ mmol m}^{-2} \text{ 23 days}^{-1}$ or 58% of the total net loss of N in the P + N container kept in light (55.2 mmol m^{-2}).

According to the above calculations about 40--60% of the inorganic N was assimilated by the microphytobenthos. The rest (40--60%) disappeared through other processes, mean daily loss being $1\text{--}1.5 \text{ mmol m}^{-2} \text{ day}^{-1}$. Denitrification may account for much of this loss, but we do not know the rate of this process in our experiments. Kaplan et al. (1979) found denitrification rates of $1\text{--}5 \text{ mg m}^{-2} \text{ h}^{-1}$ (about $1.7\text{--}8.5 \text{ mmol m}^{-2} \text{ day}^{-1}$) in salt marshes, while Nakajima et al. (1981) found a rate of $30 \text{ mmol m}^{-2} \text{ day}^{-1}$ in a eutrophicated pond. They estimated that only a third of the inorganic N was taken up by algae, the rest being denitrified.

Phytoplankton



Sediment

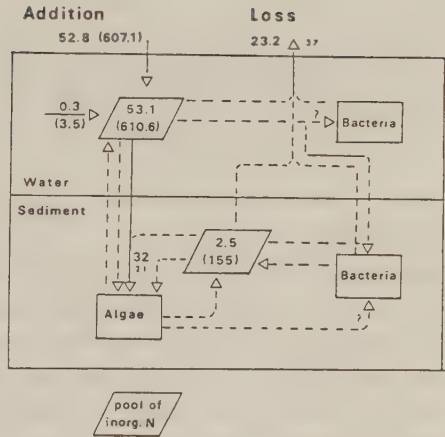


Fig. 8. Budget for inorganic nitrogen ($\text{NO}_3 + \text{NO}_2 + \text{NH}_4\text{-N}$) for the phytoplankton and sediment (sieved) containers with additions of P + N calculated from carbon fixation during the 23-day laboratory experiment. For phytoplankton container N content is expressed as $\cdot 10^{-6}\text{M}$ and for sediment container as mmol m^{-2} , concentrations expressed as $\cdot 10^{-6}\text{M}$ are shown in brackets; small numerals (21, 37) show the uptake and loss of N respectively calculated from the difference between uptake in the containers kept in light and in the dark.

Uptake rates in our containers with sieved and unsieved sediment (Fig. 7, SIEVED/UNSIEVED) were similar, suggesting that the activity of macrofauna did not affect the uptake rate, probably because the low density.

The N concentration decreased more rapidly in the containers with material from the eutrophicated Lomma Bay than in the Falsterbo canal containers. Since we found no distinct increase in microbenthic growth in the Lomma sediment we can fairly safely assume that denitrification was proportionately higher in Lomma Bay than in the Falsterbo canal. Denitrification rate in sediment has been shown to be proportional to the concentration of nitrate in the overlying water (e.g. Nedwell, 1982).

Release of P could be observed after only a few days in the container with Lomma sediment kept in the dark, but not until after 20 days in that with Falsterbo sediment (Fig. 7). Anoxic conditions develop fast in the sediment of eutrophicated areas because of the high amounts of organic matter present.

CONCLUSIONS

Microbenthic algae have an ability to remove nutrients from the water column, although not as effectively as phytoplankton. They also seem to be important controllers of the nutrient flux (P and N) between sediment and water. By producing oxygen they keep the sediment surface oxygenated thereby decreasing the transport of P to the overlying water.

Our observations are thus a supplement to those of Henriksen et al. (1980) who concluded that the microphytobenthos is an important controller of the nitrogen flux between the sediment surface and the overlying water.

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REFERENCES

- Admiraal, W. 1977: Tolerance of estuarine benthic diatoms to concentrations of ammonia, nitrite ion, nitrate ion and orthophosphate. - *Mar. Biol.* 43: 307-315.
- Admiraal, W., Peletier, H. and Zomer, H. 1982: Observations and experiments on the population dynamics of epipelagic diatoms from an estuarine mudflat. - *Est. Coast. Shelf Sci.* 14: 471-487.
- Atkinson, M.J. and Smith, S.V. 1983: C:N:P ratios of benthic marine plants. - *Limnol. Oceanogr.* 28: 568-574.
- Berland, B.R., Bonin, D.J. and Maestrini, S.Y. 1980: Azote ou phosphore? Considérations sur le 'paradoxe nutritionnel' de la mer méditerranée. - *Oceanol. Acta* 3, 1: 135-141.
- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. - *Neth. J. Sea Res.* 8: 260-291.
- Carlberg, R. (ed.) 1972: New Baltic manual with methods for sampling and analyses of physical, chemical and biological parameters. - ICES Coop. Res. Rept Ser. A. 29, 145 pp.
- Darley, W.M., Montague, C.L., Plumley, F.G., Sage, W.W. and Psalidas, A.T. 1981: Factors limiting edaphic algal biomass and productivity in a Georgia salt marsh. - *J. Phycol.* 17: 122-128.
- Estrada, M., Valiela, I. and Teal, J.M. 1974: Concentration and distribution of chlorophyll in fertilized plots in a Massachusetts salt marsh. - *J. Exp. Mar. Biol. Ecol.* 14: 47-56.
- Fenchel, T. and Straarup, B.J. 1971: Vertical distribution of photosynthetic pigments and the penetration of light in marine sediments. - *Oikos* 22: 172-182.
- Fleischer, S., Rydberg, L. and Stiebe, L. 1982: Transport of nitrogen and phosphorus to the Laholm Bay (in Swedish). - *Vatten* 38: 451-460.
- Gargas, E. 1970: Measurements of primary production, dark fixation and vertical distribution of the microbenthic algae in the Öresund. - *Ophelia* 8: 231-253.
- Gargas, M. and Gargas, E. 1982: Growth physiological conditions of marine microalgae in the topmost 10 cm of the sediment. - *Vatten* 38: 189-198.
- Goldman, J.C. 1976: Identification of nitrogen as a growth-limiting nutrient in waste waters and coastal marine waters through continuous culture algal assays. - *Water Res.* 10: 97-104.
- Granéli, E. 1978: Algal assay of limiting nutrients for phytoplankton production in the Öresund. - *Vatten* 36: 117-128.
- Granéli, E. 1981: Bioassay experiments in the Falsterbo Channel - nutrients added daily. - *Kieler Meeresforsch.*, Sonderh. 5: 82-90.
- Granéli, E. and Granéli, W. (in press): Eutrophication and dinoflagellate blooms in Swedish coastal waters - possible causes and countermeasures.
- Granéli, E., Edler, L., Granéli, W. and Fleischer, S. (in press): Possible causes of changes in the fluctuation and succession of phytoplankton, leading to red tide on the Swedish west coast. - *Oceanologica Acta*.
- Haardt, H. and Nielsen, G.A. 1980: Attenuation measurements of monochromatic light in marine sediments. - *Oceanologica Acta* 3: 333-338.
- Harrison, W.G., Azam, F., Renger, E.H. and Eppley, R.W. 1977: Some experiments on phosphate assimilation by coastal marine plankton. - *Mar. Biol.* 40: 9-18.
- Henriksen, K., Hansen, J.I. and Blackburn, T.H. 1980: The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. - *Ophelia*, Suppl. 1, 249-256.
- Hoek, van den, C., Admiraal, W., Colijn, F. and de Jonge, V.N. 1979: The role of algae and seagrasses in the ecosystem of the Wadden Sea: A review. In Wolff, W.J. (ed.) *Flora and Vegetation of the Wadden Sea*, 3: 1-206. Rotterdam.

- Jeffrey, S.W. and Humphrey, G.F. 1975: New spectrophotometric equations for determining chlorophylls a, b, c₁ and c₂ in higher plants, algae and natural phytoplankton. - *Biochem. Physiol. Pflanzen (BPP)* 167: 191-194.
- Kaplan, W., Valiela, I. and Teal, J.M. 1979: Denitrification in a salt marsh ecosystem. - *Limnol. Oceanogr.* 24: 726-734.
- Lorenzen, C.J. 1967: Determination of chlorophyll and phaeopigments: spectrophotometric equations. - *Limnol. Oceanogr.* 12: 343-346.
- Nakajima, M., Nishimura, H. and Kumagai, M. 1981: Dynamics of phosphorus and nitrogen during algal blooms in a controlled ecosystem. - *Verh. Internat. Verein. Limnol.* 21: 263-267.
- Nedwell, D.B. 1982: Exchange of nitrate and products of bacterial nitrate reduction, between seawater and sediment from a U.K. saltmarsh. - *Estuar. Coast. Shelf Sci.* 14: 557-566.
- Raalte, C.D. van, Valiela, I., and Teal, J.M. 1976a: The effect of fertilization on the species composition of salt marsh diatoms. - *Water Res.* 10: 1-4.
- Raalte, C.D. van, Valiela, I. and Teal, J.M. 1976b: Production of epibenthic salt marsh algae: Light and nutrient limitation. - *Limnol. Oceanogr.* 21: 862-872.
- Sakshaug, E., Andresen, K., Mykkestad, S. and Olsen, Y. 1983: Nutrient status of phytoplankton communities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition. - *J. Plankt. Res.* 5: 175-196.
- Sullivan, M.J. 1981: Effects of canopy removal and nitrogen enrichment on a *Distichlis spicata*-edaphic diatom complex. - *Estuar. Coast. Shelf Sci.* 13: 119-129.
- Sullivan, M.J. and Daiber, F.C. 1975: Light, nitrogen and phosphorus limitation of edaphic algae in a Delaware salt marsh. - *J. Exp. Mar. Biol.* 18: 75-88.
- Sundbäck, K. 1983a: Seasonal variation in microphytobenthic primary production and chlorophyll a content on a sandy coast with shallow brackish water, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. 1983b: The species composition of benthic diatom assemblages on a sandy coast with shallow brackish water, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. 1983c: Spatial variation in chlorophyll a content and species composition of diatom assemblages in relation to sediment characteristics in a non-tidal shallow bay, S Öresund, Sweden. - Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. and Persson, L.-E. 1981: The effect of microbenthic grazing by an amphipod, *Bathyporeia pilosa*, Lindström. - *Kieler Meeresforsch. Sonderh.* 5: 573-575.
- Thomas, W.H. 1970: On nitrogen deficiency in tropical Pacific oceanic phytoplankton: photosynthetic parameters in poor and rich water. - *Limnol. Oceanogr.* 15: 380-385.

THE EFFECT OF GRAZING BY AN AMPHIPOD, *BATHYPOREIA*
PILOSA Lindström ON MICROPHYTOBENTHIC BIOMASS
AND PRIMARY PRODUCTION *

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ABSTRACT

Two laboratory experiments in which different densities of the amphipod *Bathyporeia pilosa* Lindström were allowed to graze on natural epipsammic microflora (diatoms), showed that natural amphipod densities limited the chlorophyll a content and primary production (^{14}C assimilation).

The species composition of the diatoms was studied in the sediment, in the stomach contents and in the faecal pellets. In the one experiment there was selection for the genus *Cocconeis*, while no clear selectivity was found in the other experiment. We consider that the degree of selectivity by the amphipod is dependent on the size, availability and relative abundance of *Cocconeis* on the sand grains.

INTRODUCTION

It has often been suggested that grazing controls the biomass of the microphytobenthos in shallow waters. For instance, drops in microbenthic chlorophyll *a* peaks have been interpreted as a reflection of overgrazing (Cadée and Hegeman, 1974; Taasen and Høisaeter, 1981; van Es, 1982). Some experimental investigations have confirmed this (Pace et al., 1979; Branch and Branch, 1980), but on the other hand grazing has also been found to stimulate primary production (Hargrave, 1970; Cooper, 1973).

The benthic microflora of exposed and semi-exposed sandy substrates in shallow brackish water in Öresund (the Sound) consists mainly of small (c. 10 µm) epipsammic diatoms (Sundbäck, 1983b; Sundbäck and Persson, 1981). The amphipod *Bathyporeia pilosa* Lindström is a dominant of the macrofauna: Nicolaisen and Kanne-worff (1969) have reported densities of up to 60,000 individuals per m². They also found that *B. pilosa* lives on the microflora of sand grains which it scrapes off with the mouth parts. The density of *Bathyporeia pilosa* has been found to vary during the year. This variation may be connected with changes in the biomass and primary production of the microphytobenthos (Persson, 1982). Our purpose was to investigate a possible relationship between *Bathyporeia pilosa* and the microbenthic algae and to study food selectivity in *B. pilosa*.

MATERIAL AND METHODS

Experiment A was run from 26 November 1979 to 11 January 1980 (winter), Experiment B from 22 May to 1 July 1980 (early summer) (Table 1). The top layer (1 cm) of sediment was collected from a shallow semi-exposed sandy bottom on the shore of the central part of Öresund (Fig. 1): mean depth c. 0.5 m, salinity c. 10 ‰. The sand was sieved to remove macrobenthic animals (mesh size 0.5 mm), and washed by shaking it in filtered seawater (4--6 times) to remove detritus and unattached organisms. The amphipods were collected separately from the top layer by sieving and in each experiment four densities of *B. pilosa* were introduced into cylindrical plexiglass aquaria (ID 10 cm) containing a 5 cm layer of washed sand and 400 ml of filtered seawater from the sampling

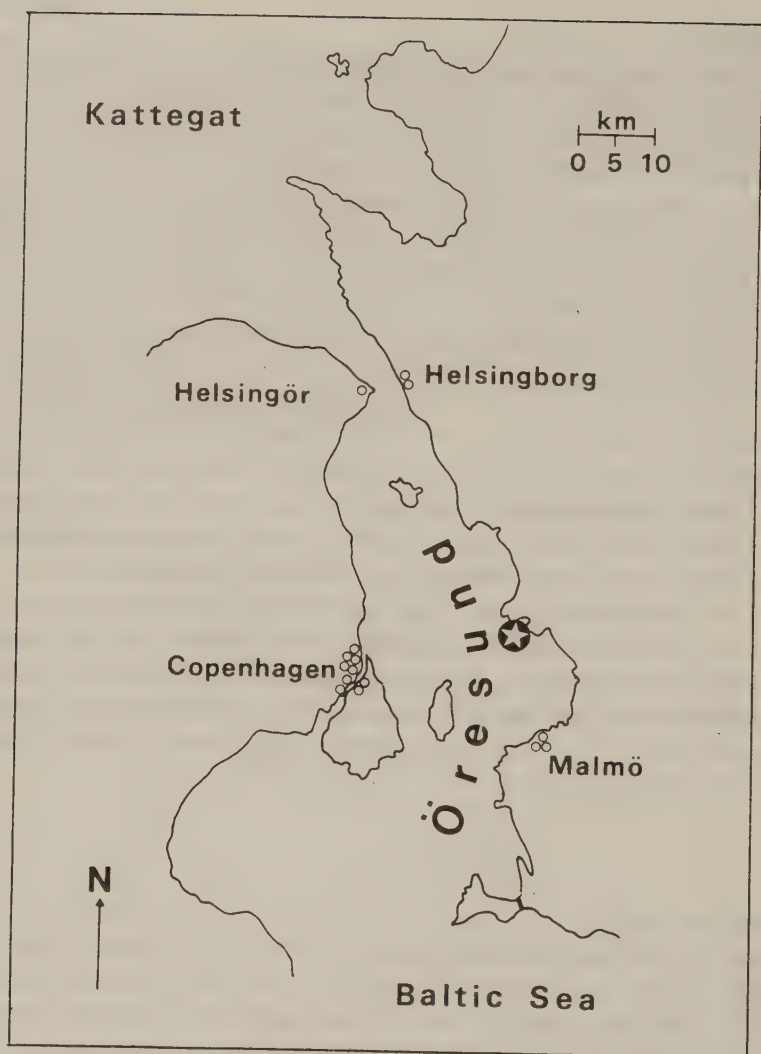


Fig.1. The sampling site in Öresund.

Table 1. Experimental design: Number of *Bathyporeia* introduced into the aquaria (I-V) in Experiments A and B (in brackets the corresponding number as indiv. m⁻²) and the natural density at the sampling site.

Experiment	Aquaria in experiments					Sampling site
	I	II	III	IV	V	
Expt A	25	100	114	220	0 *	
79-11-26	(c. 3,100)	(c. 12,500)	(c. 14,250)	(c. 27,500)	(0)	c. 7,500
--80-01-11						
(winter)						
Expt B	8 *	28	63 *	130 *	0 *	
80-05-22	(c. 1,000)	(c. 3,560)	(c. 7,800)	(c. 10,200)	(0)	c. 4,000
--80-07-01						
(summer)						

* duplicate aquaria used

site. In each experiment duplicate control aquaria (V) without *Bathyporeia* were used. In Experiment B duplicate aquaria were also used for all four densities (I--IV). The aquaria were incubated in a water-bath (16--18°C) placed under a skylight. The grazing effect of *B. pilosa* was estimated by measuring changes in chlorophyll a content (Lorenzen, 1967) and primary production (¹⁴C uptake; see Sundbäck, 1983a) in the top 0.5 cm of the sand in the aquaria one a week. Samples were taken with a cut-off 2 ml plastic syringe (ID 5 mm) used as a piston sampler. from the sampling site. In each experiment duplicate control aquaria (V) without *Bathyporeia* were used. In Experiment B duplicate aquaria were also used for all four densities (I--IV). The aquaria were incubated in a water-bath (16--18°C) placed under a skylight. The grazing effect of *B. pilosa* was estimated by measuring changes in chlorophyll a content (Lorenzen, 1967) and primary production (¹⁴C uptake; see Sundbäck, 1983a) in the top 0.5 cm of the sand in the aquaria once a week. Samples were taken with a cut-off 2 ml plastic syringe (ID 5 mm) used as a piston sampler.

To study the species composition of diatoms in the sand (before and after washing), in the stomach contents and in faecal pellets, we prepared permanent slides for diatom counts (see Sundbäck, 1983b). One series of slides was prepared before beginning the experiments, one halfway through, and one when the experiments had been concluded. C. 500 diatom valves were counted on each slide and the relative abundance (RA) was expressed in per cent. Selectivity was determined using Ivlev's (1961) electivity index ranging from -1 to +1. In Experiment B mortality in *B. pilosa* at different population densities was determined both for the introduced adults and juveniles that had hatched during the experiment. Inorganic nutrients ($\text{PO}_4\text{-P}$, $\text{NO}_3\text{+NO}_2\text{-N}$ and $\text{NH}_4\text{-N}$) were quantified in the overlying water at the end of Experiment B using the standard methods described in Carlberg (1972).

RESULTS

Both experiments showed the same general trend, i.e. an increase in chlorophyll a content and primary production with decreasing numbers of *Bathyporeia pilosa* (Fig. 2). This tendency became evident after one week in Experiment A and after three weeks in Experiment B. In both experiments there was a decrease in the microphytobenthic biomass at natural densities of *B. pilosa* (c. 7,500 indiv. m^{-2} in Experiment A and 4,000 indiv. m^{-2} in Experiment B). However, in the second experiment primary production was slightly stimulated at the lowest density of animals (Fig. 2).

The slight increase in chlorophyll a content at the initially highest density of *B. pilosa* (130) towards the end of Experiment B (34--40 days) was probably caused by the decrease in grazing pressure owing to high mortality (see below).

The microflora of unwashed sand consisted of small (mean cell length c. 10 μm) epipsammic pennate diatoms. Flat diatoms, such as *Achnanthes hauckiana* Grun., *A. lemmermannii* Hust. and small *Navicula* spp., made up 75--85% when based on cell counts. The relative abundance of the species was not affected by washing the sand (Tables 2 and 3). About 60 species were identified (see Appendix).

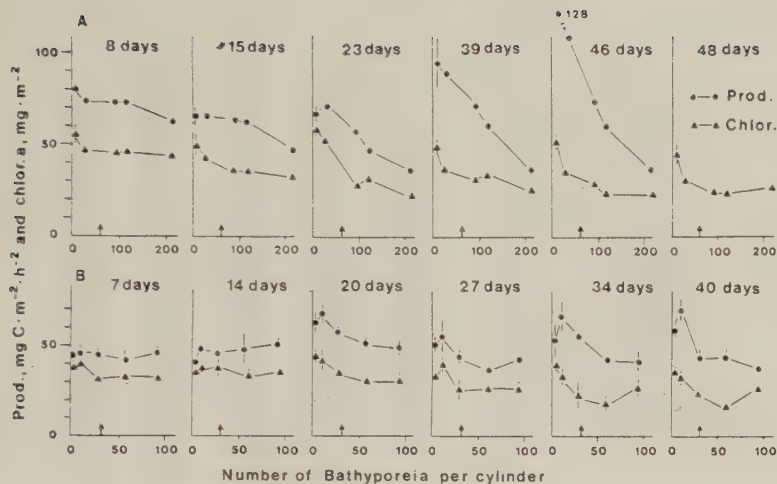


Fig. 2. The effect of varying densities of *Bathyporeia pilosa* on the primary production and chlorophyll *a* content of the microbenthic flora in Experiments A and B. Vertical lines show the range of values in two duplicate aquaria. Arrows indicate the natural density of *B. pilosa* at the sampling site. From Sundbäck and Persson, 1981.

In Experiment A about half the diatoms ingested by *Bathyporeia pilosa* were species of *Cocconeis*, mainly *C. scutellum* Ehrenb. (Table 2; Fig. 3). If based on cell volume the proportion is even higher since the *Cocconeis* species found were larger than the other species. The electivity coefficient (Ivlev, 1961) was 0.61--0.84. No preference was shown for *Cocconeis* species (or any other species) in Experiment B (Fig. 3; Table 3). No difference was observed between adults and juveniles (Table 3). There were more broken diatom valves in the stomach contents and faecal pellets than in the sand. This suggests that the feeding behaviour of *B. pilosa* affected the diatom frustules mechanically. Towards the end of Experiment B cyanophytes (cf. *Lyngbya* sp.) were observed. Since only cleaned diatoms were counted we do not know if the cyanophytes were included in the diet of *B. pilosa*. However, blue-green algae have been reported as being poor food sources for grazers (Hargrave, 1970; Kofoed, 1975; Nicotri, 1977).

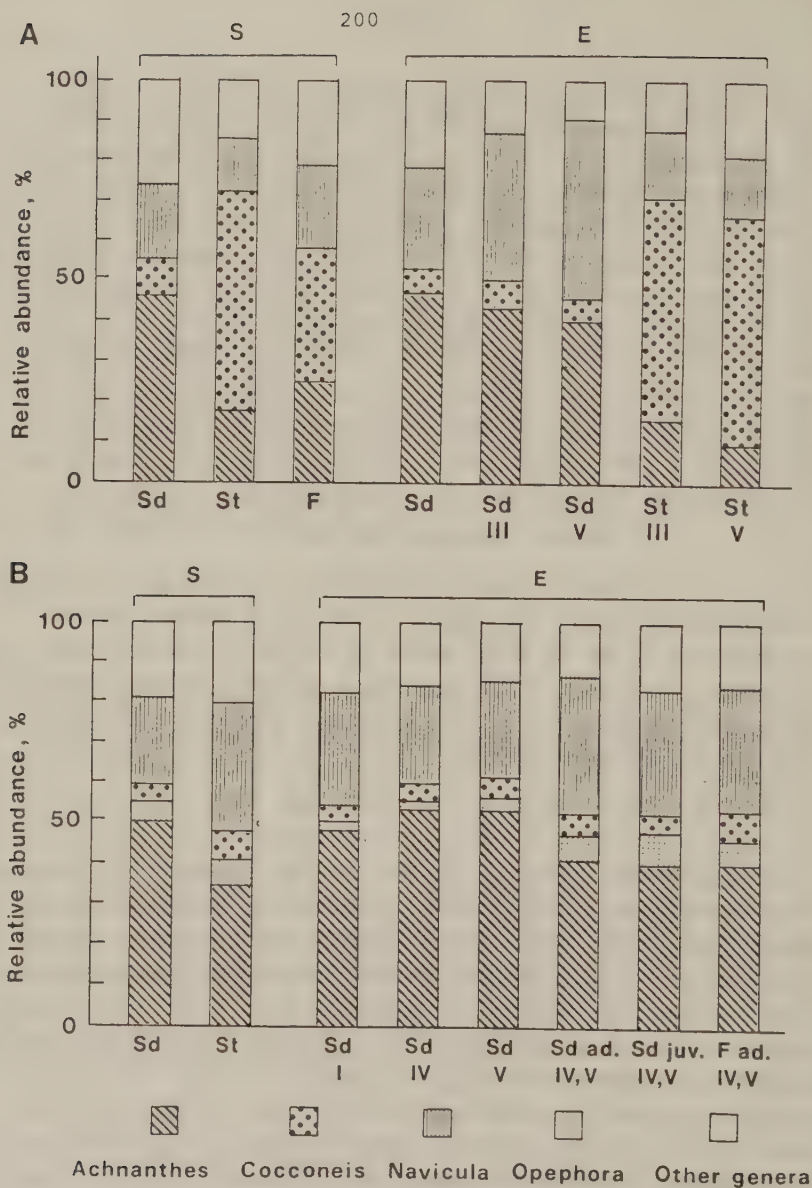


Fig. 3. The relative abundance of the commonest diatom genera in the sand (Sd), and in the stomach contents (St) and faecal pellets (F) of *Bathyporeia pilosa* in Experiments A and B, S at the sampling site and E at the end of the experiment. Roman numerals denote the different aquaria (see Table 1).

Table 2. Experiment A. Relative abundance of the commonest diatom species ($RA > 5\%$) at the sampling site (a) and in aquarium IV at the end of the experiment (b).

(a) Sand (unwashed)		<u>Stomach contents</u>		<u>Faecal pellets</u>	
Achnanthes hauckiana	15.5	Cocconeis scutellum	33.0	Cocconeis scutellum	20.1
A. lemmermannii	13.1	C. placentula	19.3	Achnanthes hauckiana	16.8
A. delicatissima	8.0	Navicula sp. 1	7.5	Cocconeis placentula	10.4
Navicula sp. 1	7.8	Achnanthes hauckiana	7.3	Navicula sp. 1	10.1
Achnanthes cf. tenera	5.3	A. lemmermannii	5.4	Achnanthes lemmermannii	5.9
				Opephora martyi	5.4
(b) Sand (washed)		<u>Stomach contents</u>			
Navicula sp. 1	19.6	Cocconeis scutellum	34.7		
Achnanthes hauckiana	16.7	C. placentula	18.9		
Navicula sp. 3	9.8				
A. lemmermannii	8.9				
Navicula cryptocephala	6.1				
A. delicatissima	5.6				
A. cf. tenera	5.3				

Table 3. Experiment B. Relative abundance of the commonest diatom species (RA > 5%) at the sampling site (a) and in the aquaria II, III and IV (mean RA) at the end of the experiment (b).

(a) Sand (unwashed)	<u>Stomach contents</u> (adults)		<u>Stomach contents</u> (juveniles)		<u>Faecal pellets</u> (adults)	
Ac hauckiana	14.6	Ac lemmermannii	12.6		Ac hauckiana	14.6
Ac lemmermannii	12.9	Ac hauckiana	12.1		Na sp. 1	13.2
Ac delicatissima	11.7	Na sp. 1	10.1		Ac lemmermannii	10.4
Na sp. 1	8.9	Na sp. 3	7.1		Ac delicatissima	6.7
Ac cf. tenera	5.3	Op martyi	5.4		Na sp. 3	6.5
(b) Sand (washed)						
Ac hauckiana	19.3	Ac lemmermannii	14.5	Ac hauckiana	15.0	
Ac lemmermannii	15.9	Ac hauckiana	14.0	Na sp. 1	12.8	
Na sp. 1	9.7	Na sp. 1	13.3	Ac lemmermannii	11.1	
Ac delicatissima	6.8	Na sp. 3	7.6	Ac delicatissima	7.9	
Ac cf. tenera	5.7	Ac delicatissima	6.4	Op martyi	6.1	
Na sp. 3	5.3	Op martyi	5.9	Fr pinnata	5.3	
		Na sp. 5	5.4	Na sp. 3	5.3	

Ac = Achnanthes, Na = Navicula, Op = Opephora, Fr = Fragilaria

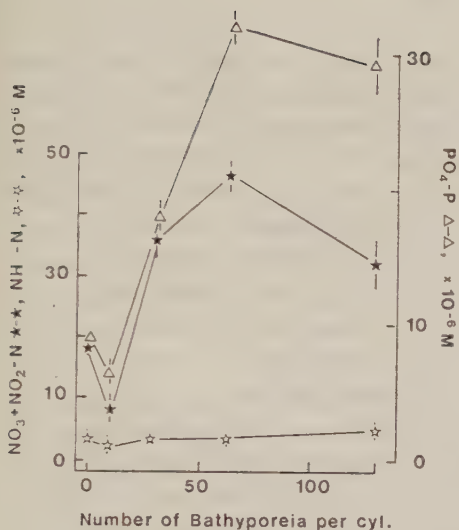


Fig. 4. The concentration of inorganic nutrients in the overlying water at the end of Experiment B. Range in two parallel aquaria indicated by lines.

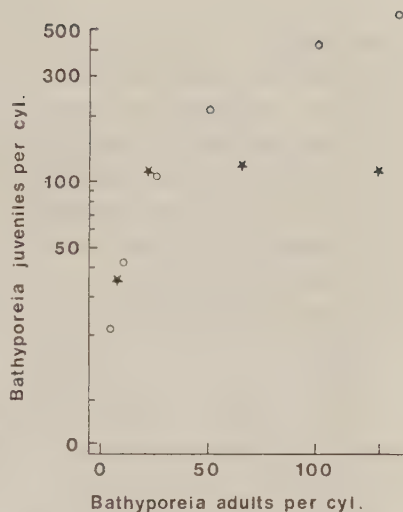


Fig. 5. Density-dependant-mortality of *Bathyporeia* juveniles in Experiment B. o theoretical output without mortality, $\star$ observed output.

At the end of Experiment B the concentrations of inorganic phosphorus and nitrogen in the overlying water were positively correlated with the density of *Bathyporeia* (Fig. 4). However, at the lowest initial density used slightly lower concentrations were found than in the control aquaria.

Juveniles that had hatched during Experiment B showed density-dependant mortality, maximum densities of c. 120 per aquarium ($=15,000 \text{ indiv. m}^{-2}$) being found in aquaria II and III (Fig. 5). The same was found true of the adults. At the lowest density, 63% of the adults survived till the end of the experiment, at the higher densities only 36--39%.

DISCUSSION

Our results indicate that *Bathyporeia pilosa* at natural population densities can limit both biomass and primary production in the epipsammic microflora. The effect of grazing was more pronounced in Experiment A (winter) as shown by the almost linear inverse relationship between animal density and chlorophyll a content (biomass) and primary production after c. 3 weeks. This is in part result of the higher animal densities used in Experiment A. It was also difficult to check the density of *B. pilosa* during Experiment B since juveniles hatched and mortality of adults varied.

Our results accord with those of Nicotri (1977), Pace et al. (1979) and Branch and Branch (1980), who found that natural densities of grazing gastropods limited the microalgal biomass (chlorophyll a content). The slight increase in primary production at the lowest animal density used (c. 1/4 of the density at the sampling site) in Experiment B agrees with the findings by Pace et al. (1979). However, our results do not support Hargrave's (1970) findings that natural densities of amphipods stimulated primary production. Both nutrient release (Hargrave, 1970) and increase in turnover rates independent of increase in rate of nutrient regeneration (Cooper, 1973) have been suggested as causes of this effect. Our measurements of inorganic phosphorus and nitrogen concentrations in the overlying water demonstrated that increase in animal density produced an increase in these nutrients (cf. Henriksen et al., 1980), but the grazing pressure proved to be more limiting. An increase in both microbenthic chlorophyll a content and primary production with nitrogen enrichment has been demonstrated for Öresund (Granéli and Sundbäck, 1983).

In Experiment A *Bathyporeia* showed strong selection for the genus *Cocconeis*, while in Experiment B the composition of the diatom flora found in the stomach contents and faecal pellets was the same as that found in the sand. In our experiments flat diatoms attached to cavities in sand grains (life form (a) in Sundbäck, 1983b) dominated the epipsammic flora. It has been suggested that the accessibility of food particles, such as is determined by strength of adhesion and general morphology influences passive selection (Nicotri, 1977; Lopez and Levinton,

1978, Medlin, 1981). According to Nicotri (1977) chain-forming moderately sized and loosely attached diatoms are most easily removed by intertidal gastropods. In our experiments the protruding cells of *Opephora martyi* and the chain-forming *Fragilaria pinnata* (life form (b) in Sundbäck, 1983b) should thus be the most readily available species though analysis of the stomach contents showed that the commonest diatom was *Cocconeis* which has been reported to be avoided by gastropods at least (Patrick, 1972; Nicotri, 1977; Medlin, 1981). This may not apply to crustaceans such as *Bathyporeia*. McElhone (1980), working with oligochaetes feeding on periphyton, found a positive correlation between ingestion and large cell volume. He suggested that selection may be determined by cell volume rather than cell shape or some other specific character.

Since we investigated selectivity on oxidized diatom samples (to enable species identification) we do not know which cells in the stomach contents and faecal pellets had been digested and this aspect needs further investigation. Nicotri (1977), in a study using gastropods, noted differential digestion of ingested diatom species. The mechanical damage to the diatom frustules that we observed in the stomach contents and faecal pellets may facilitate digestion, though it may not be necessary since digestion may be accomplished by chemical degradation (Nicotri, 1977). Frustule ornamentation (e.g. pores) may affect digestion since digestive enzymes can enter through pores.

We consider that the large numbers of *Cocconeis* cells ingested by *B. pilosa* in Experiment A could be explained by both the size and location of the cells. In Experiment A the *Cocconeis* cells were larger, the mean cell length being c. 20 μ m as against c. 10 μ m for other species present. Light microscope and scanning electron microscope observations (Sundbäck, unpublished) show that *Cocconeis* is often found in exposed positions on the sand grains thus being more readily accessible than species found in the cavities.

The lack of preference for *Cocconeis* in Experiment B may be a result of the lower relative abundance (4% as against 9%) and the smaller size (12 μ m as against 20 μ m) of *Cocconeis* cells in the sand (cf. preference for *Cymbella* in McElhone, 1980).

The density-dependent mortality found here supports the hypothesis that food limitation is a cause of seasonal migration in

B. pilosa (Persson, 1982). Direct physical interference between individuals is not likely, since the densities used were much lower than the maximum densities found in nature.

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REFERENCES

- Branch, G.M. and Branch, M.L. 1980: Competition in *Bembicium auratum* (Gastropoda) and its effect on microalgal standing stock in mangrove muds. - *Oecologia* 46: 106-114.
- Cadée, G.C. and Hegeman, J. 1974: Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. - *Neth. J. Sea Res.* 8: 260-291.
- Carlberg, S.R. (ed.) 1972: New Baltic manual with methods for sampling and analysis of physical, chemical, and biological parameters. ICES Coop. Res. Rept. Ser. A. 29: 145 pp.
- Cooper, D.C. 1973: Enhancement of net primary productivity by herbivore grazing in aquatic laboratory microcosms. - *Limnol. Oceanogr.* 18: 31-38.
- Es, van F.B. 1982: Community metabolism of intertidal flats in the Ems-Dollard Estuary. - *Mar. Biol.* 66: 95-102.
- Granéli, E. and Sundbäck, K. 1983: Response of planktonic and microbenthic algal assemblages to nutrient enrichment in shallow coastal waters, SW Sweden. - Part of doct. thesis, University of Lund, Sweden.
- Hargrave, B.T. 1970: The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. - *Limnol. Oceanogr.* 15: 21-31.
- Henriksen, K., Hansen, J.I. and Blackburn, T.H. 1980: The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. - *Ophelia*, Suppl. 1: 249-256.
- Ivlev, V.S. 1961: Experimental ecology of the feeding of fishes. Yale Univ. Press, New Haven, Conn. 302 pp.
- Kofoed, L.H. 1975: The feeding biology of *Hydrobia ventrosa* (Montagu). I. The assimilation of different components of the food. - *J. Exp. Mar. Biol. Ecol.* 19: 233-241.
- Lopez, G.R. and Levinton, J.S. 1978: The availability of microorganisms attached to sediment particles as food for *Hydrobia ventrosa* Montagu (Gastropoda: Prosobranchia). - *Oecologia* 32: 263-275.
- Lorenzen, C.J. 1967: Determination of chlorophyll and phaeo-pigments: Spectrophotometric equations. - *Limnol. Oceanogr.* 12: 343-346.
- McElhone, M.J. 1980: Some factors influencing the diet of coexisting, benthic, algal grazing Naididae (Oligochaeta). *Can. J. Zool.* 58: 481-487.
- Medlin, L.K. 1981: Effects of grazers of epiphytic diatom communities. - In Ross, R. (ed.) Proceedings of the sixth symposium of recent and fossil diatoms, pp. 399-411.
- Nicolaisen, W. and Kanneworff, E. 1969: On the burrowing and feeding habits of the amphipods *Bathyporeia pilosa* Lindström and *Bathyporeia sarsi* Watkin. - *Ophelia* 6: 231-250.

- Nicotri, M.E. 1977: Grazing effects of four marine intertidal herbivores on the microflora. - Ecology 58: 1020-1032.
- Pace, M.L., Shimmel, S. and Darley, W.M. 1979: The effect of grazing by a gastropod, *Nassarius obsoletus*, on the benthic microbial community of a salt marsh mudflat. - Est. Coast. Mar. Sci. 9: 121-134.
- Patrick, R. 1972: Benthic communities in streams. - Trans. Conn. Acad. Sci. 44: 269-284.
- Persson, L.-E. 1982: Seasonal migration of *Bathyporeia pilosa* Lindström in the southern Baltic. - Ophelia 21: 205-213.
- Sundbäck, K. 1983a: Seasonal variation in microphytobenthic primary production and chlorophyll *a* content on a sandy coast with shallow brackish water, S Öresund, Sweden.- Manuscript, part of doct. thesis, University of Lund, Sweden.
- Sundbäck, K. 1983b: The species composition of benthic diatom assemblages on a sandy coast with shallow water, S Öresund, Sweden.- Manuscript, part of doct. thesis, University of Lund.
- Sundbäck, K. and Persson, L.-E. 1981: The effect of microbenthic grazing by an amphipod, *Bathyporeia pilosa*, Lindström. - Kieler Meeresforsch., Sonderh. 5: 573-575.
- Taasen, J.-P. and Høisaeter, T. 1981: The shallow-water soft-bottom benthos in Lindåspollene, Western Norway. 4. Benthic marine diatoms, seasonal density fluctuations. - Sarsia 66: 293-316.

APPENDIX

Species list

- Achnanthes biasoletiana* (Kützing) Grunow
A. delicatissima Simonsen
A. delicatula (Kützing) Grunow
A. hauckiana Grunow
A. lemmermannii Hustedt
A. pseudosolea Simonsen
A. cf. tenera Hustedt
A. sp. 1
A. sp. 2
Amphora abludens Simonsen
A. coffeaeformis (Agardh) Kützing
A. exigua Gregory
A. holsatica Hustedt
A. ovalis var. *libyca* (Ehrenberg) Cleve
A. ovalis var. *pediculus* (Kützing) Van Heurck
A. tenerima Aleem & Hustedt
Anorthoneis excentrica (Donkin) Cleve
Catenula adhaerens Mereschkowsky
Cocconeis diminuta Pantocsek
C. peltoides Hustedt
C. placentula et var. Ehrenberg
C. scutellum Ehrenberg
C. scutellum var. *stauroneiformis* Rabenhorst
C. sp. 2
Fragilaria pinnata Ehrenberg
F. virescens var. *subsalina* Grunow
Gomphonema sp.
Lichmophora sp.
Navicula ammophila Grunow
N. amphipleuroides Hustedt
N. arenicola Grunow
N. bahusiensis Grunow
N. biskanteri Hustedt
N. cancellata Donkin
N. cineta (Ehrenberg) Ralfs

- N. crucifera* Grunow
N. cryptocephala Kützing
N. cryptolyra Brockmann
N. inserta Hustedt
N. florinae Möller
N. gracilis (Ehrenberg)
N. hansenii Möller
N. cf. incerta Grunow
N. nolens Simonsen
N. peregrina (Ehrenberg) Kützing
N. peregrina var. *meniscus* (Schumann) Grunow
N. phyllepta Kützing
N. salinarum et var. Grunow
N. subinflata Grunow
N. teneroides Hustedt
N. sp. 1
N. sp. 2
Nitzschia dissipata (Kützing) Grunow
N. frustulum var. *subsalina* Hustedt
N. pusilla Lange-Bertalot
N. sigma (Kützing) Wm. Smith
Opephora martyi Héribaud
O. schulzii Brockmann
Pinnularia ambigua Cleve
Pleurosigma angulatum (Quekett) Wm. Smith
Synedra tabulata (Agardh) Kützing

